CFTR [illegible]

milk, milk composition,
milk examination, milk
Milk adulteration, regulations,
Cream, [illegible]
Condensed milk,
milk powder,
Butter, margarine, cheese,
milk analysis, dairy

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Water analysis	H. B. Stocks

RICHMOND'S

DAIRY CHEMISTRY

revised by

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FIFTH EDITION
Based on the previous revision by
G. D. ELSDON and G. H. WALKER

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE,
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PREFACE TO FIFTH EDITION

In this edition we have restricted ourselves to the addition of new matter published up to 1951 or 1952 and have deleted some of the early work which is now obsolete on account of the development of techniques, or has been superseded by the results of later research.

We regard "Richmond" as essentially a working handbook for chemists dealing with dairy products and we have therefore not dealt exhaustively with bacteriological tests, nutritive values, and other subjects which, while intimately concerned with dairying, are not strictly speaking dairy chemistry. References to the major works on these subjects are given. The sole exception is the chapter on "Statutory tests".

We gratefully acknowledge the permission given by the Comptroller of Her Majesty's Stationery Office to reproduce the methods for the statutory methylene blue, phosphatase and turbidity tests, and by the British Standards Institution to reproduce certain techniques. We are also grateful to the Society of Public Analysts for permission to reproduce the methods described in the reports of the Analytical Methods Committee.

We are indebted to Dr R. Aschaffenburg, Mr J. Hall, Dr T. L. McMeekin, Dr R. J. MacWalter, Mr A. J. Parsons, Mr J. H. Prentice, Dr S. J. Rowland, Dr G. R. Tristram and Mr F. C. White for information and guidance on certain points, and to Miss Catherine Gilgallon and Mrs Margaret Irving for assistance in the preparation of this revision

Finally we express our thanks to Mr A. L. Barton for reading the proofs, to Miss Doris Knight for invaluable help at all stages in our revision, and to the editorial department of the Publishers for their co-operation and assistance rendered while the book was passing through the press.

J. G. DAVIS
F. J. MACDONALD

December, 1952

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PART I

GENERAL AND THEORETICAL

HAPTER 1

THE COMPOSITION OF MILK

Definition

There is no official definition of milk in Great Britain. It appears to be tacitly assumed that the meaning of the term is so familiar that no definition is necessary, but it may well be that the natural characteristic of refusing to see those problems whose solution presents difficulties has not been without influence in this connection. In the Food and Drugs Act of 1938, it is not even defined as "the substance usually known as milk". In this Act the definition of butter is given as: "The substance usually known as butter, made exclusively from milk"; so that we have the definition of one substance depending upon a term which itself is not defined. General usage of the term "milk" suggests that it refers to the liquid obtained from the udders of cows, but there is no general specification as to the breed, the number, or the general condition of the animals. The making of any official definition that was not too wide on the one hand, and which did not affect present usages on the other, would certainly be difficult.

A suggested definition (Elsdon and Walker) is: "Milk is the entire product of the complete and uninterrupted milking of milch cows which are properly cared for and in good health." The Geneva Congress has defined it as "The integral product of entire and uninterrupted milking of the female milch cow in good health and well nourished and not overworked. It ought to be collected in the proper manner and contain no colostrum." The Federal definition of the U.S.A. is: "Milk is the fresh, clean lacteal secretion obtained by the complete milking of one or more healthy cows, properly fed and kept, excluding that obtained within 15 days before and 10 days after calving, and containing not less than 8·5 per cent of solids-not-fat, and not less than 3·25 per cent of milk fat." Various other similar definitions have been suggested from time to time.

It is obvious that, apart from the necessary definition of the "secretion of the cow's udder", there are two approaches to the vexed problem of the variability of this secretion. We may attempt to eliminate the freak "milks" either by describing as accurately as we can the condition of the cow, udder and method of milking, or we can lay down compositional limits. In view of the inevitability of freak secretions, the only really satisfactory way is to prescribe limits. A possible legal definition for *market milk* (which is really what we are concerned with here) is the following—

> "Market milk is the secretion of the cow's udder containing not less than 3 per cent fat and 8·5 per cent solids-not-fat, and in which casein nitrogen constitutes not less than 75 per cent of the total nitrogen and which does not clot on boiling."

The last two properties conveniently eliminate colostrum, bad "mastitis milk" and other abnormal fluids, and also badly soured and chemically unbalanced milks. The terms "healthy", "normal", etc., cannot be defined and are therefore meaningless and should not be included in any scientific or legal definition.

Milk consists essentially of an oil–water or fat–water emulsion, the continuous phase being the aqueous one. The oil phase consists of the fat which is well known as butter fat; its properties and composition are treated in some detail below. The aqueous phase contains proteins (chiefly casein, which is present as an ultra-microscopic dispersion of particles of a compound of the protein with calcium phosphate); a sugar, lactose—the "sugar of milk" of commerce; mineral salts; and traces of other substances including vitamins, often referred to as the minor constituents, but of which some at least are of considerable importance.

All female mammals can secrete a liquid having, in general, properties similar to those of cows' milk. There are, however, considerable differences between the secretions of different mammals; these differences are considered in Chapter 4.

Average "composition"

It is by no means easy to give an absolutely fair estimate of the average composition of milk even for one country—still less for the whole world. It is obviously out of the question to examine the milk of every cow in any country at each milking on any given day, and any investigation which is substantially less thorough than this must leave some element of doubt, even if the likelihood of error be small. Yet even this would only give the average for the particular day in question, and there is undoubted evidence of seasonal variation. However, by making a number of examinations, small as compared with the total number of cows, but sufficiently large to give a reasonable idea of the likely variations, including seasonal ones, a result can be obtained which is sufficiently near the truth for practical purposes.

It has been found convenient to regard the material left by drying at 100° C as "total solids", and to subdivide the total solids into "fat" and "solids-not-fat", for which the synonym "non-fatty-solids" is sometimes used.

In this chapter the main variations will only be considered for fat and solids-not-fat (s.n.f.). The variations of the individual constituents of the solids-not-fat will be dealt with in the chapter on the constituents of milk, whilst more detailed variations will be considered in the chapter entitled "Variations in the Composition of Milk".

In an earlier edition of this book, Richmond considered the question at some length and gave his conclusions on the results published up to about the year 1920. He deduced, from about 330,000 analyses made over a period of twenty years in the laboratory of the Aylesbury Dairy Company Ltd, the average composition of milk to be—

Water	87·34	*per cent*
Fat	3·75	,, ,,
Milk-sugar	4·70	,, ,,
Casein	3·00	,, ,,
Albumin	0·40	,, ,,
Ash[1]	0·75	,, ,,
Other constituents	0·06	,, ,,
	100·00	,, ,,

---

[1] The ash is determined by heating to 500° C a weighed portion of the milk and weighing the residue. Owing to the decomposition which takes place, the nature of the constituents in the ash is not quite the same as that of the mineral constituents of the milk.

It must be clearly understood, however, that there is no such thing as an "average composition of milk"; figures obtained will be merely a function of the sources of the analyses. No two milks are ever exactly alike, and the "average" obtained will depend on the methods of analysis and on the breed, the cows concerned, the season, and many other factors. Further, from the practical point of view, distinction should be made between milk as produced from the cow and milk as sold to the public. The latter is much more uniform in composition, but there is naturally always a possibility of the presence of a certain amount of extraneous water which may have obtained access, not necessarily by intent, during the many stages of handling and treatment. Moreover, average values for the composition of milk must always be considered in relation to the method of procuring the samples, otherwise deductions may be most misleading. So many factors can affect composition that it is not surprising that average values differ somewhat. The only scientific way of dealing with the problem is to say that the average value of x determinations of fat, etc., made on milk samples as obtained from herd bulks (or as sold to the public) between the years . . . . and . . . . in England (or other geographical unit) was . . . . Any limitation of sampling (area, breed, season, type of herd, etc.) can easily lead to markedly different average values.

Further it is essential to ensure that the analytical methods used are identical, or at least give the same results. Appreciable differences in values for s.n.f., ash, etc., can be obtained according to the method used. Freak or unusual results are due to freak cows, severe mastitis, etc., and for this reason no special significance should be attached to extreme values.

The summary, in Table 1.1, of recent investigations of average values will be found useful.

Table 1.1—Average composition of milk (predominantly Shorthorn) in England and Wales

Authority . .	Richmond	Davies	Golding, Mackintosh and Mattick	Baker and Cranfield	Elsdon	Cranfield, Griffiths and Ling
Solids-not-fat	9·00	9·02	8·89	8·91	8·77	8·75
Fat	3·75	3·67	3·89	3·61	3·61	3·71
Protein . .	3·20	3·42				
Lactose . .	4·70	4·78				
Ash	0·75	0·73				
Minor organic constituents	0·35					
Total solids . .	12·75	12·69				
Number of samples . .	330,000		3,000	300,000	770,000	730
Date	Up to 1920		1932	1923–31	1903–34	1923–26

Golding *et al.* (1932) carried out investigations, extending over three years, into the composition of the milk of a typical southern herd of ten Shorthorn cows. In all, over 3,000 samples were examined, the average being 3·89 per cent of fat and 8·89 per cent of solids-not-fat. The distribution of fat and solids-not-fat in 2,135 of these samples is given in Tables 1.2 and 1.3—

Table 1.2—Fat distribution (*Golding et al.*)

Fat per cent	No. of samples	
	Morning	Evening
2·4	2	—
2·5	1	—
2·6	11	—
2·7	19	—
2·8	57	—
2·9	57	—
3·0	102	—
3·1	145	—
3·2	172	—
3·3	134	—
3·4	142	—
3·5	101	—
3·6	62	1
3·7	39	1
3·8	18	7
3·9	7	8
4·0	5	28
4·1	—	47
4·2	—	90
4·3	—	114
4·4	—	114
4·5	—	138
4·6	—	102
4·7	—	111
4·8	—	85
4·9	—	65
5·0	—	57
Over 5·0	—	93
Totals	1,074	1,061

Table 1.3—Solids-not-fat distribution (*Golding et al.*)

Solids-not-fat per cent	No. of samples	
	Morning	Evening
8·3	1	2
8·4	3	24
8·5	25	35
8·6	16	42
8·7	48	153
8·8	138	285
8·9	291	306
9·0	352	168
9·1	143	40
9·2	43	5
9·3	12	1
9·4	2	—
Totals	1,074	1,061

Table 1.4—Composition of milk (*Cranfield et al.*)

Fat		Solids-not-fat		Proteins	
Per cent	No. of samples	Per cent	No. of samples	Per cent	No. of samples
Below 2·6	11	Below 8·1	4	2·7–2·79	5
2·6–2·79	12	8·1–8·19	5	2·8–2·89	22
2·8–2·99	37	8·2–8·29	13	2·9–2·99	38
3·0–3·19	67	8·3–8·39	26	3·0–3·09	100
3·2–3·39	96	8·4–8·49	59	3·1–3·19	130
3·4–3·59	99	8·5–8·59	103	3·2–3·29	157
3·6–3·79	112	8·6–8·69	107	3·3–3·39	134
3·8–3·99	93	8·7–8·79	113	3·4–3·49	53
4·0–4·19	83	8·8–8·89	116	3·5–3·59	35
4·2–4·39	44	8·9–8·99	80	3·6–3·69	21
4·4–4·59	31	9·0–9·09	47	3·7–3·79	14
4·6–4·79	21	9·1–9·19	33	3·8–3·89	4
4·8 and above	26	9·2 and above	26	3·9–4·05	2

Very similar results are given by these same authors on a further 1,498 samples (1935). (Cf. also W. L. Davies (1933a)).

Baker and Cranfield (1933) examined the data obtained from some 300,000 samples taken from farms mainly in Cheshire, Derbyshire, Staffordshire and Leicestershire during the years 1923–1931; the average figures found were 3·61 per cent of fat and 8·91 per cent of solids-not-fat.

Cranfield *et al.* (1927) examined the milk of fifteen different herds during the years 1923 to 1926; a total of 732 samples was examined, the number of times each herd was sampled varying from 98 in the case of a Derbyshire herd of 5–8 cows, to 12 in the case of a Shropshire herd of 40 cows. The averages found were 3·71 per cent of fat and 8·75 per cent of solids-not-fat. The distribution is shown in Table 1.4.

Table 1.5—Milk fat frequencies each year from 1926 to 1936: County of Lancaster

Fat per cent	Number of samples				Percentage of total samples
	1926 to 1930	1931 to 1935	1936	Total 1926 to 1936	
Under 2·5	74	70	26	170	0·512
2·5	44	33	8	85	0·257
2·6	79	80	12	171	0·515
2·7	98	83	30	211	0·636
2·8	171	166	35	372	1·121
2·9	234	179	39	452	1·362
3·0	542	518	110	1,170	3·526
3·1	565	566	142	1,273	3·837
3·2	772	777	194	1,743	5·253
3·3	979	1,001	231	2,211	6·664
3·4	1,188	1,201	239	2,628	7·920
3·5	1,233	1,405	265	2,903	8·750
3·6	1,221	1,431	230	2,882	8·686
3·7	1,163	1,458	258	2,879	8·677
3·8	1,061	1,273	208	2,542	7·661
3·9	890	1,028	193	2,111	6·362
4·0	804	1,022	189	2,015	6·073
4·1	604	716	132	1,452	4·376
4·2	480	693	120	1,293	3·897
4·3	384	493	96	973	2·932
4·4	260	393	65	718	2·164
4·5	216	308	70	594	1·790
4·6	154	236	44	434	1·308
4·7	120	191	29	340	1·025
4·8	85	142	28	255	0·769
4·9	67	111	14	192	0·579
5·0	66	80	14	160	0·482
Over 5·0	441	433	77	951	2·866
Total samples	13,995	16,087	3,098	33,180	100·000

Table 1.6—Milk solids-not-fat frequencies each year from 1926 to 1936: County of Lancaster

Solids-not-fat per cent	Number of samples				Percentage of total samples
	1926 to 1930	1931 to 1935	1936	Total 1926 to 1936	
Under 7·5	27	15	0	42	0·126
7·5	11	3	0	14	0·042
7·6	6	5	0	11	0·033
7·7	13	8	0	21	0·063
7·8	12	14	0	26	0·078
7·9	22	21	1	44	0·132
8·0	29	33	6	68	0·204
8·1	33	67	6	106	0·319
8·2	76	108	16	200	0·602
8·3	78	180	22	280	0·843
8·4	158	261	23	442	1·332
8·5	449	672	56	1,177	3·547
8·6	763	1,455	297	2,515	7·579
8·7	1,286	2,491	384	4,161	12·540
8·8	2,009	3,161	531	5,701	17·182
8·9	2,559	3,080	649	6,288	18·951
9·0	2,487	2,293	482	5,262	15·858
9·1	1,937	1,284	312	3,533	10·647
9·2	1,172	598	180	1,950	5·877
9·3	536	227	91	854	2·573
9·4	233	73	25	331	0·997
9·5	64	23	13	100	0·301
Over 9·5	35	15	4	54	0·162
Total samples	13,995	16,087	3,098	33,180	100·000

Elsdon (1935) has published a number of figures obtained by different observers. A large proportion of the samples involved were taken under the Food and Drugs Act from many widely distributed parts of England, Wales, and Scotland and examined by Public Analysts. It should be remembered that such samples contain a proportion of adulterated samples, and that there is likely to be a preponderance of samples of morning milk, which is usually poorer in fat than evening milk. These figures will therefore be a little lower than the true average in solids-not-fat and probably appreciably lower than the true average in fat. Samples taken in the County of Lancaster under the Food and Drugs Act between the years 1910 and 1936, a total number of 75,213, were found to have an average fat content of 3·70 per cent and an average solids-not-fat content of 8·89 per cent. The frequencies for fat and solids-not-fat for the years 1926–1936, inclusive, are given in Tables 1.5 and 1.6 above.

One thousand eight hundred and fifty-four " appeal-to-cow " samples taken during the years 1903 to 1935, inclusive, were found to have an average fat content of 3·47 per cent and an average solids-not-fat content of 8·82 per cent.

Table 1.7—Average composition of milk: various districts

Area	Period	No. of samples	Fat per cent	Solids-not-fat per cent	Total solids per cent
Battersea	1934	743	3·58	8·78	12·36
Bethnal Green	1927/1934	2,354	3·45	8·79	12·24
Birmingham	1910/1934	55,318	3·61	8·75	12·36
Bolton	1920/1934	2,773	3·63	8·84	12·47
Bradford	1934	978	3·75	9·11	12·86
Bristol	1908/1934	16,573	3·55	8·80	12·35
Burnley	1920/1934	2,281	3·76	8·88	12·64
Cadbury's (Glos.)	1934	3,799	3·54	8·80	12·34
Cadbury's (Staff. and Shrop.)	1934	4,472	3·46	8·80	12·26
Cardiff	1929/1934	4,473	3·74	8·81	12·55
Chester (County)	1925/1934	7,304	3·63	8·91	12·54
*Dargie, A.	1930/1934	5,559	3·61	8·77	12·38
Derby County	1903/1934	18,343	3·63	8·73	12·36
Devon	1934	869	3·74	8·96	12·70
Durham	1917/1934	10,377	3·58	8·73	12·31
*Evans, H. J.	1934	454	3·62	8·76	12·38
Glasgow	1906/1934	18,285	3·41	8·67	12·08
Gloucester (County)	1925/1934	4,499	3·69	8·80	12·49
Gloucester (City)	1925/1934	893	3·62	8·80	12·42
Hackney	1934	589	3·51	9·00	12·51
Halifax	1931/1934	789	3·73	9·05	12·78
Hammersmith	1913/1934	647	3·65	8·79	12·44
Hereford	1931/1934	90	3·58	8·85	12·43
Hull	1909/1934	14,183	3·65	8·81	12·46
Kent	1922/1934	21,026	3·71	8·87	12·58
Lambeth	1934	778	3·65	8·81	12·46
Lancaster (County)	1910/1934	68,693	3·69	8·89	12·58
Leeds	1934	1,788	3·64	8·82	12·46
Leicester	1931/1934	3,236	3·68	8·91	12·59
Lewisham	1934	267	3·74	8·79	12·53
Liverpool	1923/1934	47,344	3·62	8·86	12·48
Manchester	1920/1934	17,422	3·48	8·91	12·39
Middlesbrough	1933/1934	3,744	3·40	8·85	12·25
Poplar	1925/1934	3,692	3·49	8·80	12·29
Portsmouth	1926/1934	4,696	3·73	8·86	12·59
Rochdale	1925/1934	2,332	3·64	8·84	12·48
Salford	1914/1934	17,886	3·62	8·84	12·46
Scarborough	1931/1934	383	3·49	8·77	12·26
Sheffield	1921/1934	10,894	3·63	8·83	12·46
Southampton	1925/1934	2,131	3·67	8·93	12·60
Southend-on-Sea	1934	215	3·38	8·94	12·32
Stafford (County)	1922/1934	13,962	3·73	8·81	12·54
Stepney	1924/1934	9,931	3·65	8·70	12·35
Stoke Newington	1934	67	3·68	8·85	12·53
*Tatlock, R. R. and Thomson, R. T.	1921/1934	9,045	3·60	8·82	12·42
United Dairies, Ltd.	1934	343,360	3·60	8·71	12·31
Wandsworth	1934	941	3·59	8·78	12·37
Warrington	1928/1934	485	3·83	8·76	12·59
Warwick	1920/1934	8,322	3·64	8·76	12·40
Westminster	1930/1934	2,377	3·71	8·82	12·53
Total averages	—	771,662	3·61	8·77	12·38

* Where names of persons are given, the figures include more than one area.

It must be remembered, however, that such samples are not random samples—they are only taken because some previous sample has been found to have a composition which raises a presumption that it is adulterated. Included in such a collection will therefore be found samples from all the herds which are known to be giving a composition below the average. Consequently, it is to be expected that the average composition of a considerable number of "appeal-to-cow" samples will be markedly below the average from all the herds from which the "appeal-to-cow" samples have been selected.

The results obtained from a total of 771,662 samples (420,031 Food and Drugs Act samples and 351,631 commercial samples) taken over the years 1903 to 1934 inclusive, have also been calculated. The average results were: fat, 3·61 per cent, and solids-not-fat, 8·77 per cent. The figures for different areas show considerable variations: thus the percentage of fat varied from 3·38 per cent in Southend-on-Sea to 3·83 in Warrington, whilst the solids-not-fat varied from 8·67 per cent in Glasgow to 9·11 in Bradford; but attention must be called to the very considerable differences between the numbers of the samples on which the averages are based. The results are given in Table 1.7.

Table 1.8—Average composition of milk: various districts 1910–1934

Year	No. of samples	Fat Per cent	Solids not-fat Per cent	Total solids Per cent
1910	2,743	3·54	8·74	12·28
1911	3,228	3·54	8·73	12·27
1912	4,276	3·62	8·80	12·42
1913	5,297	3·65	8·78	12·43
1914	5,475	3·59	8·76	12·35
1915	5,838	3·63	8·79	12·42
1916	5,437	3·65	8·79	12·44
1917	6,024	3·69	8·78	12·47
1918	7,758	3·63	8·75	12·38
1919	7,650	3·62	8·81	12·43
1920	8,041	3·66	8·83	12·49
1921	8,374	3·64	8·85	12·49
1922	10,749	3·69	8·86	12·55
1923	15,163	3·66	8·86	12·52
1924	17,058	3·69	8·83	12·52
1925	18,056	3·62	8·85	12·47
1926	19,181	3·66	8·83	12·49
1927	19,902	3·69	8·86	12·55
1928	21,118	3·63	8·82	12·45
1929	22,114	3·61	8·83	12·44
1930	22,596	3·64	8·87	12·51
1931	24,930	3·68	8·85	12·53
1932	26,192	3·65	8·82	12·47
1933	27,280	3·63	8·80	12·43
1934	384,786	3·60	8·72	12·32
Total averages ..	698,943	3·62	8·77	12·39

In Table 1.8 the annual averages are given for all the districts for which figures for the individual years are available. These figures show little variation, the percentage of fat varying from 3·54 to 3·69 and that of the solids-not-fat from 8·72 to 8·87. Again, however, the figures for the different years are not strictly comparable, as the areas included, and also the number of samples, have varied from year to year: thus the large number of samples from Messrs Cadbury and United Dairies Ltd (a total of 351,631) occur together in the year 1934.

Average values for other countries

Since breed is the major factor controlling *average* values, analyses reported from other countries may differ appreciably from those quoted above because of different breeds predominating. Thus the preponderance of Ayrshires in Scotland raises the Scottish average above that for England and Wales.

Averages obtained by Tocher (1925) from the milks of individual cows are given in Table 1.9.

Overman *et al.* (1929) examined 1,998 samples, taken at intervals of five weeks, from 198 lactations of 130 individual cows. Each sample represented all the milk produced by each individual cow during three days. The average percentage of fat was 4·45, whilst that of solids-not-fat was 9·35. The extremes were: for fat, 2·60 to 8·37 per cent; for solids-not-fat, 7·20 to 11·90.

G. Cornalba (1934) gives the average composition of Lombardy milk as: fat 3·75 per cent, solids-not-fat 8·70.

The various results dealt with above are arranged together in Table 1.10.

Recent investigations

McCance and Widdowson's " The chemical composition of foods " (1940) gives numerous compositional data for milk and dairy products. Fixsen and Roscoe (1940) have given figures for the vitamin contents of milk and milk products.

A very comprehensive and detailed account of the range of analytical data for Swedish milk has been published by Platon and Sjöström (1943). Data for milk in Switzerland are given by Kästli (1946) as follows (average values): sp. gr., 1·0322, fat 3·729 per cent, solids-not-fat 9·044 per cent, and total solids 12·773 per cent. Bakalor (1947) has reported that the average total solids of milk sent to condensing factories in S. Africa was 12·17 per cent (11·99 to 12·63) with a fat value of 3·59 per cent (3·41 to 3·92). Caulfield *et al.* (1939) gives the following data for three-day composites of American cows (averages in brackets)—

	Fat	*Solids-not-fat*
Holstein ..	3·1–4·1 (3·69)	8·1–9·1 (8·52)
Ayrshire ..	3·6–5·0 (4·32)	8·4–9·5 (8·91)
Guernsey ..	4·0–6·3 (5·09)	8·4–10·0 (9·29)
Jersey	4·8–6·7 (5·53)	8·9–10·3 (9·54)

El-Sokkary and Hassan (1949) give mean values and standard errors for the milk of Egyptian cows and buffaloes which are listed in Tables 1.11 and 1.12.

Table 1.9—Average composition of milk (*J. F. Tocher*)

	Ayrshire cows	All cows
Fat	4·09 per cent	3·95 per cent
Solids-not-fat	8·75 ,,	8·80 ,,
Total solids	12·84 ,,	12·75 ,,
No. of samples	341	676

Table 1.10—The composition of milk

Author	No. of samples	Average figures	
		Fat Per cent	Solids-not-fat Per cent
Golding *et al.*	3,000	3·89	8·89
Baker and Cranfield ..	300,000	3·61	8·91
Cornalba	—	3·75	8·8
Ayanyi	5,452	3·67	8·83
Cranfield *et al.* ..	732	3·71	8·75
Overman *et al.* ..	1,998*	4·45	9·35
MacDougal	12,000	3·82	—
Richmond	330,000	3·78	8·74
Elsdon†	771,662	3·61	8·77
Farrington and Woll‡	—	3·7	8·9
van Slyke§	5,552	3·9	9·0
König	—	3·7	9·0
Davies	—	3·73	8·89
Tocher	676	3·95	8·78
Crowther	4,220	3·7	8·78

* Mostly Guernsey-Holstein cows.
† Reported by.
‡ *Testing Milk and its Products*, Madison, U.S., 1916.
§ *Modern Methods of Testing Milks and Milk Products*, New York, 1916.

Table 1.11—Cows' milk

	Individual samples		*Bulk samples*	
Fat %	4·57	0·12	4·29	0·06
Solids-not-fat % ..	9·25	0·05	9·03	0·03
Total N%	0·557	0·008	0·509	0·005
Casein N% ..	0·442	0·006	0·405	0·005
Casein no.	79·34	0·22	79·6	0·19
Sp. gr.	1·0331	0·0002	1·0337	0·0003

Table 1.12—Buffaloes' milk

	Individual samples		*Bulk samples*	
Fat %	6·64	0·11	6·60	0·09
Solids-not-fat %	9·92	0·05	9·89	0·05
Total N%	0·616	0·007	0·621	0·007
Casein N%	0·501	0·006	0·504	0·005
Casein no.	81·34	0·18	81·2	0·31
Sp. gr.	1·0325	0·0002	1·0339	0·0004

Has the average composition of milk altered during the last 20 years?

This question has been much debated lately, and for reasons which have been discussed it is very difficult to obtain a reliable answer. Analytical data are rarely obtained under identical conditions at different times, and " fat " and " solids-not-fat " are in practice defined by analytical methods. In this country the Gerber method is practically universal for routine and the Röse-Gottlieb method for reference fat tests. Similarly the Richmond lactometer formula is practically universal for routine, and drying at 100° for reference solids-not-fats tests. Changes in methods or in techniques may easily affect results. Even if one technique is used throughout, the accuracy of the method depends on the calibration of the apparatus, and reproducibility on constancy of all errors in both method and apparatus.

Rowland (1948) has reviewed the trends in composition and expressed the opinion that fat and solids-not-fat have fallen steadily for about 20 years. Since 1941 late winter milk especially has been low in solids-not-fat. Provan and Jenkins (1949) have also discussed the problem and quoted figures from United Dairies Ltd, which suggested a fall in average s.n.f. from 8·9 per cent in 1923 to 8·6 per cent in 1946, and a slight fall in fat which may not be significant.

Davis (1952a) has recently collected data for *consumer* milk over the period 1900 to 1950 and summarises his conclusions as follows—

London	Slight fall in fat.
North-western	Rise and fall in s.n.f.
North-eastern	Constant.
Midland	Rise and slight fall in s.n.f.
Southern	Marked rise and fall in fat; less marked rise and fall in s.n.f.
Wales	Fall in fat and (possibly) s.n.f.
Scotland	Marked rise in fat.

The weighted average values for England are given in Table 1.13.

For general reviews of the question see Aschaffenburg and Rowland (1952) and Aschaffenburg and Rook (1949).

Although considerable caution must be exercised in giving judgment on this matter, the authors are of the opinion that the balance of evidence suggests that there has been a fall in average solids-not-fat values, especially since 1939, and which has been most pronounced in January–March (see also p. 73). There is, however, no reliable evidence to suggest that fat has fallen.

Table 1.13

Period		*No. of samples*	*Weighted average values*	
			Fat	*Solids-not-fat*
1900–05	..	3,415	3·61	8·61
1906–10	..	6,511	3·53	8·70
1911–15	..	21,686	3·60	8·78
1916–20	..	27,860	3·66	8·83
1921–25	..	34,448	3·65	8·91
1926–30	..	43,695	3·70	8·83
1931–35	..	61,160	3·67	8·81
1936–40	..	93,376	3·65	8·79
1941–45	..	90,078	3·64	8·76
1946–50	..	139,661	3·62	8·74

Nutritive aspects

For the very wide field of nutritive aspects of milk and milk products the reader is referred to the article on " Nutritive value of dairy products " by Kon and Henry in the 2nd edition of Davis' *Dictionary of dairying* and the following reviews: Kon and Henry (1951), (1949), Kon (1943), (1940), (1938), (1936), (1934). See also Kon and Mawson (1950).

CHAPTER 2

THE CONSTITUENTS OF MILK

In the previous chapter a list has been given of the major constituents of milk. In general the remarks refer to cows' milk—the differences between this and the milks of other species will be noted in separate sections. These constituents will now be considered in greater detail under individual headings, viz.: (1) proteins, (2) carbohydrates, (3) mineral matter, (4) fat, and (5) minor constituents (vitamins, enzymes and " extractives ").

(1) PROTEINS

From time to time quite a number of proteins have been described as being contained in milk—different authors have given numbers varying from one to ten. This wide discrepancy can be accounted for by the great difficulty in separating bodies of this class, and by the fact that the naturally occurring proteins may be broken down or modified either by decomposition in the milk itself or by the action of the reagents by which they are separated. It is now certain that there are at least three different proteins or protein groups in milk, viz.: casein, lactalbumin, and lactoglobulin. One or two others may occur in comparatively small amounts, but much uncertainty exists and no definite proof of their existence has been found.

Nomenclature

The nomenclature of the proteins in milk is rather confused, largely because of the fact that the names have been applied to cover a mixture of proteins, and also because the term β-lactoglobulin was applied by Palmer in America to that protein which is commonly called albumin in this country. Palmer designated this a globulin because it requires traces of salt to dissolve it. True albumins are considered to be soluble in pure water.

The present (1952) position may be summarised as follows:

(i) Casein is probably a mixture of three proteins, namely, α-, β- and γ-casein. A proteolytic enzyme appears to be closely associated with one of these.

(ii) Albumin is a mixture of which the chief component is that protein termed β-lactoglobulin in America.

(iii) Globulin consists of two proteins, namely eu-globulin and pseudo-globulin.

(a) Total proteins

A vast amount of work has been done on the quantitative aspect of the protein content of milk. As both casein and albumin—which together contain over 90 per cent of the nitrogen in milk—each contain 15·68 per cent nitrogen, it might be assumed that the percentage of total proteins could be obtained by multiplying the percentage of nitrogen by 6·38. There is, however, a small amount of non-protein nitrogen present in milk, amounting on the average to about 0·03 per cent. The figures for total protein usually given are therefore a little on the high side. Richmond considered that the factor 6·38 is too low and that 6·39 or 6·40 gives results nearer the truth, but later investigators have suggested a figure as low as 6·34.

Other methods have been suggested for the determinations of total proteins. In Ritthausen's method the protein and fat are precipitated together by means of copper sulphate and sodium hydroxide solution; the fat is removed by extraction with ether and the residue is weighed as proteins and ash, the ash being subsequently determined and subtracted. In Steinegger's "aldehyde figure" method, as modified by Richmond, the acidity developed by treating the milk with formaldehyde is determined under standard conditions. The "aldehyde figure" is not infrequently used as a rapid sorting test, but Ritthausen's method is now little used as it is liable to several errors.

The average amount of total protein in milk has been given as from 3·1 to 3·5 per cent, although figures as far apart as 1·66 and 8·65 have been reported. The quantity present will depend upon several factors, of which breed, age and period of lactation are probably the most important. Channel Island stock usually yields milk which contains about 3·9 per cent, a similar amount being present in the milk of Devon cattle; the milk of most other breeds contains about 3·2 to 3·5 per cent. Van Slyke has found that, in general, the amount of proteins in milk increases as lactation advances, the increase for the period being of the order of 0·6 per cent. This is supported by Tocher (1926). In human milk the change is in the opposite direction. The amount of protein in colostrum is very high—it may be as high as 25 per cent—but the high figure is largely due to the presence of far higher proportions of globulin than usual.

From the analysis of a large number of milks Vieth has deduced that the ratio between lactose, protein and ash is very nearly 13 : 9 : 2 (Vieth's ratio). This ratio gives results which are very near to the truth for the mixed milk of herds having solids-not-fat between 8·4 per cent and 9·2 per cent. It does not hold for unwatered milks having a percentage of solids-not-fat outside this range, or for the milks of individual cows.

Richmond (1920) found that the ash could be deduced from the formula: ash $= 0{\cdot}36 + 0{\cdot}11$ protein. Sherman (1903) modified this formula to: ash $= 0{\cdot}38 + 0{\cdot}10$ protein.

Timpe has suggested that the percentage of proteins can be calculated from the fat by the formula: proteins $= 2 + 0{\cdot}35$ fat. Richmond's results show this formula to be quite inaccurate, although Richmond, as well as other observers, agree that the proteins tend to be higher when the fat is higher (cf. Tocher 1926).

(b) Casein

Some confusion has arisen owing to the fact that the term "casein" has been used indiscriminately for the nitrogenous precipitates obtained from milk by different methods. It is now known that the products differ in composition according to the method of preparation, and that all differ from the substance naturally present in milk.

Casein exists in milk as a colloidal suspension containing calcium both bound to the protein molecule and associated with it as tricalcium phosphate $Ca_3(PO_4)_2$. Precipitation by acid results in removal of all or most of the calcium, whereas the precipitation by rennin (as in cheesemaking) leaves the calcium attached to the protein.

Our present knowledge of the proteins of milk is far from complete, though much work has been done on the subject. This is due to the fact that, as mentioned above, it is extremely difficult to obtain these compounds in anything like a state of purity. The method of crystallisation, which is so largely depended upon in working with other bodies, is only available for albumin, and as proteins are altered in their essential properties by very many reagents, the choice of methods of purification is limited. The difficulty is further increased by the peculiar behaviour of casein in retaining calcium salts, once it has been brought into contact with them, as is the case in milk. The proteins have been prepared in as pure a state as possible by the general method of precipitating them by some reagent, dissolving them, re-precipitating them as many times as may be thought necessary, and, finally, by eliminating such impurities as may have been introduced during the process. As there is no means of knowing when all the impurities have been eliminated, it is possible that we are yet unacquainted with the proteins of milk in a state of purity. This should not be forgotten during their study.

Halliburton has given the name " caseinogen " to the protein as actually existing in milk, restricting the word "casein" to that produced by rennin. Van Slyke states that " ' Casein ' or ' free casein ' is the base-free or uncombined protein; ' calcium casein ' or ' calcium caseinate ' is the neutral compound that is believed to be present in fresh, normal milk, consisting of casein in combination with about 1·5 per cent of CaO; ' basic calcium casein ' (or caseinate) is the compound consisting of casein in combination with about 2·5 per cent of CaO. ' Calcium paracasein ' (or paracaseinate) is the insoluble compound formed by the action of rennin on calcium casein; ' paracasein ' or ' free paracasein ' is the base-free or uncombined protein." When speaking of various conditions of this protein it is very desirable that no confusion should be allowed to arise. It is suggested that the name " casein " be used to describe the protein as present in milk, " rennin casein " and " acid casein " being used to describe the substances separated by rennin and acid respectively.

The preparation of free casein

(*a*) *Pure casein.* Many variations of method have been suggested for the preparation of free casein. The following is that due to Crowther and Raistrick (1916a). Freshly-drawn milk is centrifuged (a super-centrifuge may be an advantage) for the removal of fat; it is then diluted with three times its volume of water and heated to about 40° C. Saturated potash-alum solution is then added in sufficient quantity to produce complete precipitation. After standing for half an hour the solution is decanted from the precipitate, which is then strained through muslin and washed three times by vigorously stirring with water. The precipitate is then suspended in water, dissolved in the minimum quantity of 1 per cent solution of caustic soda, and the solution thoroughly extracted with ether and filtered. The filtered solution is then diluted with water and the casein re-precipitated with 1 per cent acetic acid, added with constant and thorough stirring. (Van Slyke carries out the first, as well as subsequent precipitations, with acetic acid and dissolves the precipitate in dilute ammonia instead of using caustic soda. The use of ammonia is not very suitable in those cases where nitrogen determinations have to be carried out on the product, and caustic soda tends to split off nitrogen and phosphorus. Moir has proposed the use of borax.) After settling, the supernatant liquid is siphoned off and the precipitate washed by sedimentation. The process of dissolving in alkali and re-precipitating is repeated at least five times.

The final solution is filtered quite clear through fine paper pulp, diluted, and precipitated with the minimum amount of 1 per cent hydrochloric acid. After washing and draining, the casein is dried by grinding, first with pure alcohol, then with pure ether and finally, if necessary, *in vacuo* over sulphuric acid at a temperature of not over 50° C. The final product should be a white dusty powder, practically free from ash.

(*b*) *Commercial casein.* On the commercial scale, casein is precipitated by one of three methods—with acids such as sulphuric, hydrochloric or acetic, with rennet, or by spontaneous souring. A separated milk is always employed which should have as small a percentage of fat as possible. The reaction is carried out in pans fitted with steam pipes, so that the milk can be brought to the desired temperature. The precipitated curd is washed with water, which is then removed as far as possible by centrifuge or filter-press. The resulting curd is broken up and dried in an oven, the temperature of which is controlled so that the drying can be carried out as rapidly as possible without the casein becoming discoloured.

The properties of free casein

Free casein is a white, fine, dusty powder, having a specific gravity of about 1·26. It is soluble in water only to the extent of about 0·1 per cent; it is insoluble in alcohol, ether, and the ordinary organic solvents, but readily soluble in dilute alkalis. Casein behaves both as an acid and as a base, although its acidic properties are somewhat more pronounced. Its solutions in alkalis have a lævo-rotation. Casein is also dissolved by alkaline earths and such alkaline salts as phosphates and borates. From these alkaline salts the casein is re-precipitated by acids. The iso-electric point is about pH 4·6.

When milk is treated with acids or acid salts, the casein is precipitated as a heavy white solid in more or less flocculent form, depending on the conditions of treatment. When milk turns sour, the lactic acid formed produces the same effect as the addition of any other acid. At ordinary temperatures precipitation occurs when the acidity reaches about 0·55 per cent expressed as lactic acid (pH 5·0 to 5·1). Increase of temperature causes precipitation to take place on the addition of less acid. When the casein of milk is precipitated by acids, the first action of the acid is to combine with the calcium of the calcium casein, forming calcium lactate and free casein. When more acid is present than is required to combine with the calcium of the calcium casein, the insoluble phosphates of the milk are changed to mono-calcium phosphate.

The composition of casein

Apart from the elements carbon, hydrogen, oxygen and nitrogen, casein contains phosphorus and sulphur. The elementary composition of "free casein" has been given by van Slyke and Bosworth (1913) as—

	Per cent
Carbon	53·50
Hydrogen	7·13
Oxygen	22·14
Nitrogen	15·80
Phosphorus	0·71
Sulphur	0·72
	100·00

The casein of the milk of different animals has been examined, but the differences found by the same observers are not so great as the differences found by different observers for the elementary composition of cows'-milk casein. Although the milks of some species show considerable variations in the physical condition of the protein clot, no differences have been observed in the chemical reactions of the respective caseins, and serological tests have produced no distinguishing features.

Eilers *et al.* (1947a) have summarised the most recent work on the composition of casein and this is given in Table 2.4 on page 26.

Carpenter (1931) has produced evidence to show that the molecular weight of casein is between 75,000 and 100,000, but free casein may be a mixture of proteins having different molecular weights, figures as high as 375,000 for some of these having been suggested by Svedberg *et al.* (1930). Casein is heterogeneous according to Mellander (1939) who found that it was composed of three electrophoretic components: α, β and γ in order of decreasing mobility. The γ-casein resembles the alcohol-soluble low-phosphorus casein of Osborne and Wakeman (1918a). Recent molecular weight estimates for casein vary from about 30,000 to 100,000. Nitschmann and Guggisberg (1941) conclude that casein particles are rod-shaped with an axial ratio of 8·7 and length of 290 Ångström units. The properties of casein, however, vary with the method of preparation. The fall in viscosity of casein solutions is enzymatic. The enzyme cannot be removed but is destroyed by heating to 80° for ten minutes at pH 8·6.

The stability of the calcium-caseinate system appears to be due to the protective colloid action of one of the components which is less than 20 per cent of the total. One gram of casein can combine with 8·8 ml of 0·1N NaOH and 7·0 ml of 0·1N HCl. Casein exerts its greatest buffering powers at pH 6 to 7 and from 10 upwards.

Coagulation of milk by rennin

When a solution of rennin is added to milk over a certain range of temperature coagulation follows in a short time, the actual time depending upon the strength of the preparation, the temperature, and the pH of the milk. Rennin, rennet, or chymase is an enzyme secreted by young mammals into their digestive tracts and is usually obtained from the fourth stomach of the calf. There is little pepsin present when the animals are very young, this enzyme appearing as development proceeds. The optimum temperature of coagulation is 41°, the optimum pH for the enzymic change 5·4. An elaborate method for the testing of rennin has been worked out by van Dam (1912), but it is usually only necessary to adopt some arbitrary standard and to compare other preparations with this. In the Monrad test, 5 ml of the rennet extract are measured into a 50 ml flask and diluted to the mark. 160 ml of milk are placed in a beaker and heated to 29° to 30° and a few small particles of straw dropped in. The rennet dilution is brought to the same temperature and 5 ml are then allowed to run quickly into the milk, which is stirred at the same time. When half the contents of the pipette have been discharged, a stop-watch is started, and the milk having been given a strong rotary motion with the stirrer or thermometer, this last is withdrawn, and the watch stopped the moment the straw floats cease to move, indicating coagulation. In this way, some dilution being taken as the standard, other extracts can be standardised by making the same dilution and comparing the times of coagulation. The reactive strengths of the extracts are then given in terms of the time of coagulation.

Another simple method is to place 25 ml milk in a boiling tube, add 1 ml of rennet (suitably diluted) and repeatedly form a film on the side of the tube by raising a glass rod. The end-point is the appearance of floccules on the glass. When testing rennet (or conversely the clotting power of milks) the temperature must be controlled to ± 0·1°.

Although various workers have attempted to prepare synthetic " milks " for the standardisation of rennet strength, these are usually not constant in behaviour because of slow changes in the state of the protein and the bound Ca–soluble Ca equilibrium. Cheesemakers commonly use bulk milk for the testing of rennet.

The composition of commercial casein

The composition of commercial casein depends to a considerable extent on the method of preparation. A considerable amount of mineral matter occurs in rennet casein, very little in acid casein. A. Burr (1910) gives the average composition of rennet casein as—

	Per cent
Water	10·38
Fat	1·89
Casein	79·45
Ash	6·51
Other ingredients	1·77
	100·00

For caseins prepared by himself he gives the factor for nitrogen to casein as from 6·35 to 6·41, the ash in four samples of acid casein as 0·0, and for four samples of rennet casein as 5·0 to 8·55.

The amount of casein present in milk

The casein of normal milk represents 79–80 per cent of the total nitrogen, so that the amount of casein can be roughly computed from the figures given above for total nitrogen calculated as proteins. A number of investigations have however, been made into the actual amount of casein present. The figures found for this determination by different observers are by no means identical, but it is probable that the differences are due, at least in part, to the varying methods of analysis adopted. The average amount of casein is of the order of 2·85 per cent. The average amount present has been variously reported as from 2·5 per cent (van Slyke) to 3·4 per cent (Oliver). Richmond gives the figures for English cattle as 3·0 per cent, whilst Tocher for Scottish cattle finds 2·4 per cent although it is probable that Tocher's figures are 0·3 per cent too low as he allowed his casein precipitate to stand overnight in contact with the precipitating acid. In milk from individual cows, Tocher found the casein to vary from 1·64 per cent to 4·14 per cent, whilst van Slyke found 1·59 per cent to 4·49 per cent. The most recent comprehensive study of this aspect is that of Rowland (1938) and Rowland and Zein-el-Dine (1938) (1939) who have studied the nitrogen distribution in normal and different types of abnormal milk. Casein N accounted for 79·2 to 80·4 per cent of the total N (average values for breeds) in normal milk and 70·3 to 74·1 per cent in infected (mastitis) milk. Average casein N values of 477, 469, 429 and 427 mg per cent were obtained for normal Guernsey, Ayrshire, Shorthorn and Friesian milk, respectively, and values of 486, 371, 390 and 354 for infected samples. In colostrum the amount of casein is of the order of 4·5 per cent, but this does not account for the whole of the great difference in the amount of total proteins.

(c) Albumin or β-lactoglobulin

Crowther and Raistrick (1916b) showed that lactalbumin from either milk or colostrum is very different in composition from the serum albumin of ox blood. The protein of whey soluble in saturated magnesium sulphate or half-saturated ammonium sulphate is commonly referred to as "albumin" but this fraction contains a number of proteins, some of which have enzymatic properties. Palmer (1934) prepared a crystallised protein from this fraction which is now named β-lactoglobulin (Cannan *et al.* 1942).

The molecular weight is about 39,000 and it is stable from pH 1 to 9. Its isoelectric point is 5·19.

Osborne has given the molecular weight of albumin as 14,796, but Sjögren and Svedberg (1930) have given evidence to suggest that it is not homogeneous, having constituents with molecular weights varying from 12,000 to 25,000. These authors state that the material in milk from which precipitated albumin is formed has a molecular weight of about 1,000, and that the high figure of the final product is caused by condensation of the material of lower molecular weight originally present in the milk.

When prepared in a state of purity, albumin is a white powder, amorphous and tasteless. In aqueous solution, 90 to 95 per cent is precipitated on heating to 70°. It can be prepared in a crystalline condition if its aqueous solution is saturated with pure magnesium sulphate, diluted with an equal quantity of water, a little acetic acid added, and the whole allowed to stand. It is not precipitated by saturating the solution with magnesium sulphate, but the further addition of acetic acid causes coagulation. It is precipitated by saturating its solution with ammonium or sodium sulphate or by means of a solution of tannic or phosphotungstic acid.

The preparation of pure albumin

Crowther and Raistrick (1916b) prepared pure lactalbumin by the method of Pinkus (1901). The combined filtrates from the globulin separation (*vide infra*) are heated to 40°, saturated with pure anhydrous sodium sulphate (free from chloride) and allowed to stand at 40° for a few hours. The precipitated albumin is then filtered off through a jacketed filter at 40°, crystallisation of the salt being prevented. The precipitate is dissolved in cold water, the solution filtered, and the filtrate saturated with magnesium sulphate. Any precipitate is removed by filtration and the filtrate again saturated with anhydrous sodium sulphate at 40°. This process is repeated four times, or once more after saturation with magnesium sulphate produces no precipitate. The final precipitate is dissolved in water, the solution filtered, and the albumin precipitated with pure alcohol, after which it is dried with pure alcohol and pure ether at a low temperature. (Cf. Woodman 1921.)

The amount of albumin present in milk

Owing to the different methods which have been used for the determination, some uncertainty exists as to the amount of albumin present in milk. Some observers, such as Richmond, Oliver, Willoughby and Abderhalden, give averages of 0·4 to 0·5 per cent whilst others, such as van Slyke, Fleischmann and Tocher, give averages of about 0·7 per cent. Many of the figures given for the albumin content of milk include the globulin. Rowland (*loc. cit.*) finds that normal milk contains on the average 9·2 per cent of its N as albumin, corresponding to 0·31 per cent as protein.

(d) Globulin

Lactoglobulin is coagulated when its solution is heated to 72°. It is not coagulated by rennin and it is soluble in acidified solutions of sodium chloride. It is precipitated by neutral sulphates, tannic acid, etc., and is therefore usually returned along with albumin. Crowther and Raistrick describe two fractions: *eu-globulin*, which is insoluble in water and in a 0·06 per cent solution of sodium chloride but soluble in a 0 6 per cent solution of sodium chloride; and *pseudo-globulin*, which is soluble in water but which is precipitated by the addition of alcohol to its aqueous solution.

Crowther and Raistrick showed that lactoglobulin obtained from either milk or colostrum is probably identical with the serum-globulin of ox-blood. This has been confirmed by other writers.

Preparation of pure globulin. The filtrate from the preparation of casein (see p. 17) is neutralised with dilute caustic soda solution and saturated with anhydrous magnesium sulphate. The precipitated globulin is filtered off on a large Buchner funnel. The precipitate is dissolved in a dilute solution of magnesium sulphate, filtered through pulp, saturated with magnesium sulphate, and the globulin filtered off as before. The precipitation is repeated four times, the filtrates being reserved each time (if required) for the preparation of the albumin. The final solution is dialysed, first in running water and then in relays of distilled water, until free from sulphates. The eu-globulin is rendered insoluble and is then filtered from the solution of pseudo-globulin, dissolved in a 0·6 per cent solution of sodium chloride, filtered, and re-precipitated by diluting with ten times its volume of distilled water. The precipitate is allowed to settle, washed by decantation, first with distilled water, and afterwards with pure alcohol, and then with pure ether. The pseudo-globulin is precipitated from its aqueous solution by means of alcohol, filtered, and the precipitate washed with alcohol and ether.

The amount of globulin present in milk

The amount of globulin present in milk is small. Crowther and Raistrick isolated only 0·03 per cent; Osborne and Wakeman (1918b) only 0·05 per cent. Davies suggests a figure of 0·2 per cent but this would seem to be definitely on the high side. The figure seems to vary considerably but appears to be in general about 0·1 per cent. Rowland (*loc. cit.*) reports an average value of 3·3 per cent of the total N or 0·11 per cent globulin.

Nitrogen distribution

By this term is meant the proportion of the total nitrogen in the various protein and other fractions. Rowland (*loc. cit.*) describes methods for determining the N distribution in milk and gives the following values for normal milk:

Table 2.1

	As % of total N			*"Protein" %*
	Min.	*Max.*	*Mean*	*Mean*
Casein	77·3	80·7	78·5	2·63
Albumin	7·5	10·7	9·2	0·31
Globulin	2·4	4·2	3·3	0·11
Proteose	2·8	5·3	4·1	0·13
Non-protein	3·7	6·4	5·0	0·16

Other workers have obtained figures as follows (Menefee *et al.*, 1941; Beach *et al.*, 1941).

Table 2.2—Nitrogen distribution in milk

Nitrogen	Cows' milk		Human milk	
	Mg/100 ml	% of total N	Mg/100 ml	% of total N
Total	540	—	162	—
Non-protein[a] ..	30	5·5	36	22
Casein[b]	430	79·5	49	30
Whey protein[c] (total)	80	15·0	77	48
Globulin[d] ..	19	3·5	—	—
Albumin[e] ..	43	8·0	—	—
Proteose[f]	18	3·0	—	—

[a] Not precipitated by 15 per cent trichloroacetic acid.

[b] Precipitated by acetic acid, pH 4·7.

[c] Nitrogen value of the casein filtrate (whey) minus non-protein nitrogen.

[d] Precipitated from casein filtrate by saturation with magnesium sulphate, pH 7·0.

[e] Obtained by subtracting globulin nitrogen plus non-protein nitrogen from the total nitrogen of the casein filtrate.

[f] Nitrogen content of filtrate from heat-coagulated milk minus non-protein nitrogen.

The electrophoretic method is more accurate than the nitrogen distribution method. Data for whey proteins are given in Table 2.3.

Table 2.3—Composition of whey from cows' milk as shown by electrophoresis.[a]

Component	Conc., %	Mobility × 10^{-5}
(*a*) Euglobulin[b]	6	—1·7
(*b*) Pseudoglobulin[c]	4	—2·5
(*c*) Component	18	—3·6
(*d*) Component	12	—4·5
(*e*) β-lactoglobulin	55	—5·1
(*f*) Component	5	—6·4

[a] Determined in veronal buffer, pH 8·4, ionic strength 0·1, with protein concentration 1·2 per cent.

[b] Insoluble in 0·3 saturation with ammonium sulphate and insoluble in water at isoelectric point in absence of salt.

[c] Insoluble in 0·3 saturation with ammonium sulphate but soluble in water at isoelectric point in absence of salt.

By ultracentrifuge methods three boundaries are obtained with whey, the α component being Kekwick's lactalbumin, the β component Palmer's β-lactoglobulin and the γ component the classical globulin.

Recent investigations on milk proteins

(*a*) *Physico-chemical aspects.* (*i*) *Casein*

Eilers *et al.* (1947a), (1947b), have reported a comprehensive study on colloidal aspects of skim milk in which casein naturally is the main interest. Tricalcium phosphate is linked with calcium caseinate and is completely detached at pH 5·2 (the pH at which casein begins to precipitate at room temperature). The degree of hydration is 40 to 50 per cent and 1 g of the dry complex occupies 3·1 ml in milk. The viscosity of milk is closely correlated with the casein and solids-not-fat contents. Heat affects viscosity initially by its effect on the casein complex and then later by producing a denaturation of the water-soluble proteins. Küntzel and Doehner (1947) postulate a theory based on spherical micelles of hydrated aggregates with more disperse particles in true solution.

Leviton and Haller (1947) in their studies of skim milk find that the average particle size is 80 mμ and the size-distribution asymmetric. Lotmar and Nitschmann (1941) have shown by high-scattering methods that the particles of casein are spherical and have a maximum size of 120 mμ. Addition of alkali results in a dispersing effect and possibly an elongation of the particles. Further physico-chemical studies on casein are reported by Nitschmann and Guggisberg (1941).

Burg (1947) has also produced some evidence that in the casein-calcium phosphate complex the protein calcium is attached to the ester phosphate of the casein, and the calcium and phosphate groups of the salt to the carboxyl and lysine amino groups, respectively, in the protein. Ramsdell and Whittier (1944) separated the protein complex by super-centrifuging and found it to consist of 4·8 per cent tricalcium phosphate and 95·2 per cent calcium caseinate.

The latter contained 1·18 per cent calcium, but Horst (1947) in reviewing the experimental work on the casein–calcium phosphate complex concludes that the salt has no fixed composition and that the method of linkage is still unknown.

De Kadt and Minnen (1947) have shown by ultra-centrifuging techniques that about 0·55 g water is bound with 1 g casein, the usual value reported. Sandelin (1946) has emphasised the role of ionic Ca in precipitating casein, especially in forming " skin " at low temperatures. Denaturation readily occurs when casein is dried at high temperatures and this may explain the variable results reported by different investigators.

Nitschman and Lehmann (1947) have shown that acid casein contains two fractions α and β which may be distinguished by electrophoresis. Rennin splits α-casein into two further fractions α_1 and α_2. Warner (1944) has separated α- and β-caseins and studied them electrophoretically and Gordon *et al.* (1949) have given data for the amino acid composition of α- and β-caseins. Hipp *et al.* (1952) have described two methods for separating α-, β- and γ-caseins.

Kamal and Turner (1951) have studied electrophoretically both iodinated and ordinary casein. Whereas ordinary casein consists of an α and a β component the iodinated casein is homogeneous.

Mellander (1947) has reported a comprehensive investigation on the differences between bovine and human casein, the latter containing only half as much phosphorus. The α-caseins in both cases have a lower N/P ratio than the original protein. Human casein is digested more slowly than the bovine.

Posternak and Pollaczek (1941) have isolated phosphopeptones from casein by pancreatic digestion. They conclude that each phosphoric group is attached to serine, and the terminal position of these complexes lends stability to the phosphopeptones. An interesting technique has been used by Cohen (1943) who has obtained four water-soluble proteins and an insoluble residue by the dry grinding of casein. The former contained more phosphorus and less tryptophane than the original casein. Rennet produced only a soft clot from one of them.

Heinemann and Parker (1951) have devised an ingenious method for determining the non-casein protein in skim milk. The protein is allowed to form a monolayer film on the surface of oxidised piston oil on a 0·2 per cent acetic acid solution. They claim that the method gives results which are in good agreement with the ordinary micro-Kjeldahl procedure.

(*ii*) *Whey-soluble proteins*

Ferry and Oncley (1941) have found that crystalline lactoglobulin is a highly polar molecule of an elongated ellipsoidal shape hydrated to the extent of about 30 per cent. Bull (1946) considers that the molecule of β-lactoglobulin (m.w. 35,000–44,000) is split under certain conditions to give a molecule of m.w. of about 17,000. He has also suggested that the molecule consists of two parallel layers of peptide chains, hydrophilic on the outside and hydrophobic on the inside. Li (1946) has also produced evidence for the non-homogeneity of β-lactoglobulin. Electrophoresis at pH 4·8 and 6·5 indicated the presence of three components. This has also been confirmed by McMeekin *et al.* (1948).

(*b*) *Chemical composition*

Brand *et al.* (1945) have given β-lactoglobulin the empirical formula

C_{1864} H_{3012} N_{468} S_{21} O_{576}, m.w. 42,000, and Cannan *et al.* (1942) conclude that one molecule contains 34 amino, 58 carboxyl, 6 imidazole and 6 guanidino groups. Chibnall (1942) suggests that the β-lactoglobulin molecule probably consists of polypeptide chains held together by carboxyl groups. Brand and Kassell (1942a) consider that the arginine content of 2·87 per cent is evidence for the minimum molecular weight as 42,000. There are 364 amino acids including 21 threonine, 15 serine, 4 to 6 histidine, 31 to 36 lysine residues and 32 amide groups in the molecule. This also contains 9 methionine, 4 cysteine, 8 half-cysteine, 9 tyrosine and 4 tryptophane residues. Of the 468 N atoms, 100 are not α-amino (1942b).

Table 2.4—Amino acid constitution of casein

	Chibnall Per cent	Eilers		
		Per cent	Molecular weight	Per cent of total nitrogen
Glutamic	13·4	21·8	147	13·2
Leucine	7·2	12·1	131	8·2
Proline	8·2	8·0	115	6·2
Valine	4·6	7·2	117	5·4
Lysine	10·1	7·2	146	8·7
Tyrosine	3·1	6·4	181	2·9
Alanine	3·2	5·6	89	5·6
Serine	5·0	5·2	105	4·3
Arginine	8·3	4·4	174	9·0
Aspartic	4·5	4·1	133	2·7
Phenylalanine	3·5	3·9	165	2·1
Histidine	5·6	3·7	155	6·3
Threonine	3·4	3·5	119	2·6
Methionine	2·1	3·2	149	1·9
Phosphoric		2·7	98	—
Oxyproline	1·5	2·1	131	1·4
Ammonia		1·6	17	0·3
Tryptophane	1·2	1·4	204	1·2
Isoleucine	3·6	1·4	131	1·0
Diamino-trihydroxydodecanic		0·75	288	0·4
Glycine	0·5	0·5	75	0·6
Cystine	0·3	0·4	240	0·3
Galactose		0·3	180	—
n-valine		0·2	117	0·2

Continuing their studies of the electrophoresis of milk proteins Stanley *et al.* (1951) have found that the composition of the components could be affected by the method of preparation. They obtained iso-electric point values of 4·8 for the albumin and β-lactoglobulin fractions. These are lower than those reported in the literature (pH 5·2).

Electron microscopy of β-lactoglobulin crystals has been described by Dawson (1951) and the structure of β-lactoglobulin crystals discussed by Riley (1951). These very recent data give a molecular weight of about 35,600, and it appears that about 85 per cent of the available free volume in the air-dry crystal is occupied by water.

Recent determinations of the amino-acid composition of casein and lactoglobulin are summarised in Tables 2.4, 2.5 and 2.6.

Table 2.5—Amino-acid constitution of proteins

	CASEIN						β-LACTOGLOBULIN			
	(as % N)						(as % N)			
	(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(e)	(g)
Alanine ..		3·5						6·1		
Arginine ..			3·6	4·1	3·7				2·9	3·2
Cysteine ..						1·1				
Cystine ..						2·29				3·5
Glutamic acid			21·4	22·5						
Histidine ..			2·6	4·1	3·2				1·7	1·8
Isoleucine ..		3·6	6·0	8·0						6·4
Leucine ..		7·2	9·5	10·8						17·0
Lysine ..			7·6	8·5	7·6				10·4	9·9
Methionine ..				3·1		3·22				
Phenylalanine		3·6	5·3	5·1				2·3		5·2
Proline ..		8·1						4·3		
Serine ..	5·9			6·8			4·1			
Threonine ..	4·6		4·1	4·5			5·1			5·8
Tryptophane				1·0		1·94				1·9
Tyrosine ..			6·5	5·4		3·78				4·2
Valine ..		4·5	7·1	7·8	6·9			4·5	5·9	7·6
N						15·60				15·53
S						1·60				1·68

(a) Rees (1946).
(b) Tristram (1946).
(c) Hier *et al.* (1945).
(d) Baumgarten *et al.* (1946).
(e) Guirard *et al.* (1946).
(f) Brand and Kassell (1942b).
(g) Bolling and Block (1943).

Gordon *et al.* (1949) have reported complete amino acid analyses of casein and α- and β- casein (Table 2.6).

Beach *et al.* (1941) have given analyses for proteins of bovine and human milk as in Table 2.7.

Tristram (1946) has described in detail a partition chromatographic method for the determination of mono-amino acids in proteins.

Meneffee *et al.* (1941), have studied the effect of different types of processing on the proteins in milk. In evaporated milk the coagulation of all the albumin and most of the globulin caused an apparent increase in the "casein fraction" and the soluble N increased. Homogenisation was without effect.

Table 2.6—Amino acid composition of caseins
g/100 g protein

	Whole casein	α-casein	β-casein
Total N	15·63	15·53	15·33
Total P	0·86	0·99	0·61
Amino N	0·93	0·99	0·72
Glycine	2·7	2·8	2·4
Alanine	3·0[a]	3·7[a]	1·7[a]
Valine	7·2	6·3	10·2
Leucine	9·2	7·9	11·6
Isoleucine	6·1	6·4	5·5
Proline	11·3	8·2	16·0
Phenylalanine	5·0	4·6	5·8
Cystine	0·34	0·43	0·0—0·1
Methionine	2·8	2·5	3·4
Tryptophane	1·2	1·6	0·65
Arginine	4·1	4·3	3·4
Histidine	3·1	2·9	3·1
Lysine	8·2	8·9	6·5
Aspartic acid	7·1	8·4	4·9
Glutamic acid	22·4	22·5	23·2
Amide N	1·6	1·6	1·6
Serine	6·3	6·3	6·8
Threonine	4·9	4·9	5·1
Tyrosine	6·3	8·1	3·2
Total	115·8[b]	115·7[b]	117·4[b]

[a] These values are provisional.

[b] Total includes amino acids, amide N calculated as ammonia, and phosphorus calculated as phosphoric acid.

Table 2.7

	Casein		*Whey protein*	
	Cow	*Human*	*Cow*	*Human*
Arginine	3·79	3·31	3·46	5·18
Cystine	0·26	0·73	2·49	3·09
Histidine	1·81	1·80	1·41	1·13
Lysine	6·20	5·21	7·91	5·80
Methionine	3·10	2·19	2·73	1·91
Tryptophane	1·11	1·69	1·81	2·32
Tyrosine	6·01	6·11	4·70	5·20
Total N	14·50	14·62	14·20	13·73
,, S	0·796	0·682	1·262	1·298
Cystine S	0·069	0·195	0·662	0·825
Methionine S	0·666	0·471	0·586	0·410

Other milk proteins

Sørensen and Sørensen (1942) have isolated from whey (i) a green lactomucin of low solubility, (ii) a red fraction of high solubility, (iii) Palmer's globulin, and (iv) a gelatinous compound. Dills and Nelson (1942) have isolated from cows' milk a protein containing 0·19 per cent bound Cu and 15 per cent N.

Weinstein *et al.* (1951) have shown that a minor protein from skim milk is capable of being photosensitised to produce typical sunlight flavour. Analytical data for this protein are given. Electrophoretic analysis indicates that the fraction has at least two components.

E. L. Smith (1948) has reviewed the immune proteins (globulins) of milk and colostrum.

Amino acids

The symposium on " Amino acids " in 1949 resulted in the publication of a useful series of papers on proteins and amino acids which adequately summarises our present knowledge in this field.

The contributions were by Dodds (1950) (introductory), Lea (1950) (the " browning reaction "), Dyson and Bavin (1950) (protein hydrolysates), Bailey (1950) (amino acids in proteins), Synge (1950) (chromatographic analysis) and Partridge (1950) (displacement chromatography for preparation).

For surveys of recent work on proteins see Tristram (1949) and McMeekin and Polis (1949).

The composition of colostrum

The main differences between the compositions of colostrum and of milk are the smaller percentage of lactose and the considerably higher percentage of proteins. In extreme cases the percentage of total proteins may approach 25. The amount of casein may be twice as high as that present in normal milk—up to 5 per cent—but the amount of globulin may be very much higher, figures as high as 12 per cent or more having been obtained. The amount of albumin is usually about twice that in normal milk. Crowther and Raistrick (1916b) give the nitrogen distribution in colostrum as follows—

Table 2.8—Nitrogen in colostrum (per cent)

No. of milking	Total nitrogen	Casein nitrogen	Albumin nitrogen	Globulin nitrogen	Non-protein nitrogen
1	2·40	0·75	0·14	1·32	0·19
2	2·01	0·68	0·17	1·02	0·14
3	1·44	0·59	0·14	0·59	0·12
4	0·97	0·51	0·11	0·31	0·04
5	0·76	0·46	0·07	0·20	0·03
6	0·75	0·46	0·06	0·20	0·03
7	0·69	0·42	0·06	0·18	0·03
8	0·65	0·46	0·05	0·12	0·02

Engel and Schlag (1924) give analytical data for colostrum as shown in Table 2.9.

Table 2.9—Composition and properties of colostrum (*Engel and Schlag*)

Time after calving (hr)	Fat per cent	Total protein per cent	Casein per cent	Albumin and globulin per cent	Clot on boiling	Titratable acidity (as lactic acid per cent)	Lactose per cent	Specific gravity	Freezing point (minus °C)
Immediately	5·10	17·57	5·08	11·34	+	0·414	2·19	1·067	0·605
6	6·85	10·00	3·51	6·30	+	0·324	2·71	1·044	0·555
12	3·80	6·05	3·00	2·96	+	0·252	3·71	1·037	0·566
24	3·40	4·52	2·76	1·48	+	0·243	3·98	1·034	0·575
30	4·90	4·01	2·56	1·20	+	0·221	4·27	1·032	0·570
36	3·55	3·98	2·77	1·03	+	0·225	3·97	1·032	0·570
48	2·80	3·74	2·63	0·99	+	0·216	3·97	1·032	0·580
72	3·10	3·86	2·70	0·97	—	0·225	4·37	1·033	0·575
96	2·80	3·76	2·68	0·82	—	0·207	4·72	1·034	0·555
120	3·75	3·86	2·68	0·87	—	0.191	4·76	1·033	0·575
168	3·45	3·31	2·42	0·69	—	0·202	4·96	1·032	0·570

In their studies on colostrum Moody *et al.* (1951) have found that during the transition from colostrum to milk the cream-line and viscosity both decreased, the change being most rapid during the first three milkings. These changes appeared to be related to changes in specific gravity, solids-not-fat, total protein, and the albumin–globulin fraction. The cream-line appeared to be associated with fat percentage, but viscosity, as measured by rate of flow, was independent of fat content. Neither cream-line nor viscosity appeared to be related to the casein, lactose, ash or leucocyte numbers.

Minor nitrogenous materials in milk

Apart from casein, albumin and globulin, other nitrogenous compounds occur in small proportions. This non-protein nitrogen is only about 5 per cent of the total nitrogen and is therefore of the order of 0·02 to 0·03 per cent of nitrogen when calculated on the original milk. These figures must be taken as approximate only, as it is not known with exactitude how much may be due to slight decomposition of the proteins during their separation. The substances either known or thought to occur include proteoses, peptones, creatine, creatinine, ammonia, urea and uric acid. None of them occurs in more than minute proportions, the maximum amount of any one being of the order of 0·03 per cent. They bear a similarity to the non-protein nitrogen compounds occurring in blood-serum; as yet no definite value has been ascribed to them.

Shahani and Sommer (1951) have described methods for determining and reported values for these substances. Their amount did not change in milk held for 10 days at 0–5° C under toluene.

(2) CARBOHYDRATES

Lactose

The principal carbohydrate present in cows' milk, probably the only one, is lactose, commonly called " sugar of milk " or milk-sugar. There is some evidence to show that the milks of some other mammals contain other substances.

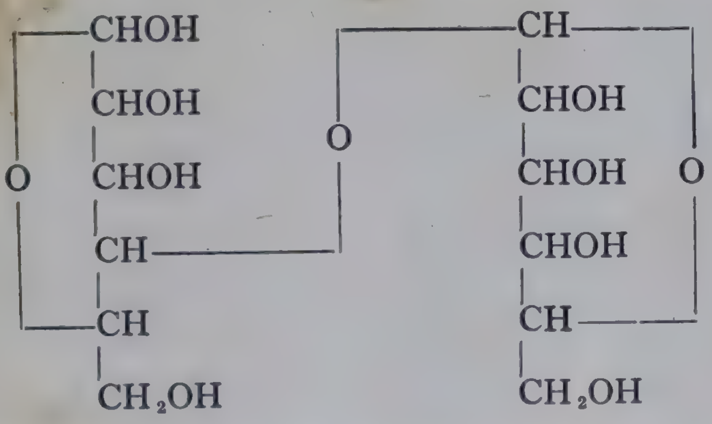
Richmond and Pappel obtained from the milk of the Egyptian buffalo a sugar distinct from lactose which they called *tewfikose*, whilst the milk of the mare is stated to undergo alcoholic fermentation, a property not possessed by lactose. Richmond and Carter are also of the opinion that the sugar of human milk is not identical with that of the cow.

On hydrolysis by acids, lactose yields a mixture of *d*-glucose and *d*-galactose. The aldehyde group of lactose is that of *d*-glucose, that of *d*-galactose being eliminated in the lactose molecule. This is shown by the reactions of several derivatives of milk-sugar; by heating milk-sugar with phenyl-hydrazine and acetic acid, phenyl-lactosazone is formed, which yields an osone on treatment with strong hydrochloric acid; this, by boiling with hydrochloric acid, yields a mixture of galactose and glucosone. By treatment again with phenyl-hydrazine, the glucosone forms phenyl-glucosazone almost immediately, and, on warming, phenyl-galactosazone is precipitated. A clear demonstration is thus afforded that the aldehyde group of the glucose only remains.

By oxidation with bromine, lactobionic acid is formed, which is hydrolysed by acids to gluconic acid and galactose; again showing that the galactose group is modified.

The reactions of milk-sugar, which are all displayed in solution, are those of an aldehyde, but from its formation of stable hydrated compounds it appears more correct to regard it as an aldehydrol.

Howarth and his collaborators have ascribed the following formula to lactose—



Anhydrous lactose occurs in two modifications—α-lactose and β-lactose. As the α-lactose also occurs as a monohydrate, lactose can exist in three modifications. The best-known modification is hydrated α-lactose, which is the ordinary milk-sugar of commerce.

Hydrated α-lactose

This is the form in which lactose crystallises from water below 94° C. Below this temperature the other two modifications change to α-lactose hydrate in the presence of water. Hydrated α-lactose exhibits muta-rotation: thus the $[\alpha]_D^{20}$ of a freshly-prepared solution is about $+ 89°$, whilst the same solution exhibits after 24 hours an $[\alpha]_D^{20}$ of $+ 55{\cdot}5°$. Heating the solution, or the addition of a little ammonia, increases the rate of change so much that the rotation becomes stable at the lower figure in a few minutes. During the change in rotation no change takes place in the density of the solution or in the molecular weight of the solute. α-lactose hydrate is practically insoluble in alcohol, ether and in most of the usual organic solvents. It is slightly but distinctly soluble in amyl alcohol on boiling, but it is probably dehydrated.

When hydrated α-lactose is dissolved in water it slowly changes into the β-form. As the latter is more soluble in water it follows that the initial solubility of the substance is not a stable figure and that it slowly increases until an equilibrium is formed, corresponding to the equilibrium of α-lactose hydrate and β-lactose; this is set up when 62 per cent has changed to the β-form. At 15° the initial solubility is such that 100 parts by weight of water dissolve 7·3 parts of α-lactose hydrate; the corresponding figure for the final solubility is 16·9. α-lactose hydrate reduces Fehling's solution and an ammoniacal solution of silver nitrate, but does not reduce Barfoed's solution. Melting-point, 202°.

Anhydrous α-lactose—It is often stated that the water of crystallisation of α-lactose monohydrate is not lost at a lower temperature than 130° C. This is not correct, as there is a slow but distinct loss at 100° C, whilst at 110° C the change is quite rapid. Anhydrous α-lactose is produced by heating the hydrated material at temperatures between 110° and 130° C. Considerable evolution of heat accompanies its solution. Melting point, 228°.

β-lactose—β-lactose is formed by crystallisation from solutions of α-lactose monohydrate at temperatures above 94°. This form, which is stable above this temperature of 94°, has a melting point 252°. Any of the three physical forms of lactose, when in solution, reach a condition of equilibrium which has been shown to be 1 part of α-lactose to 1·65 parts of β-lactose.

Commercial lactose—Commercial lactose is prepared from whey. Any remaining fat is removed in centrifugal machines and the liquid is evaporated in vacuum pans. The first crop of crude crystals obtained is recrystallised from water after the solution has been decolourised by means of charcoal.

Amount of lactose present in milk

The average lactose content of cows' milk is of the order of 4·7 to 4·8 per cent (as hydrate); the extremes recorded for the milk of individual cows are 0·68 to 6·11. The following averages have been reported—

Table 2.10—Lactose content of cows' milk

Observer	*Mean per cent*	*Extremes per cent*
Richmond	4·75	0·68 to 5·20
Overman *et al.* ..	4·86	2·41 ,, 6·11
Golding *et al.* ..	4·80	—
Cornalba ..	4·80	—
Tocher	4·64	2·70 to 5·50

Overman *et al.* have published figures for the lactose content of milks from different breeds of cattle; their results are given in Table 2.11.

The most marked effect of mastitis chemically is on the lactose content, the sugar being reduced and the salt (NaCl) content raised. When considering lactose contents, therefore, particular attention must be paid to this aspect.

Choi *et al.* (1949a) have evolved a method for the determination of α- and β-lactose in skim milk powder and whey powder. Excess of the α-hydrate is added to a known quantity of sample and the solubility measured against time.

The total initial solubility is then the initial solubility of the α- plus the quantity of the β-form. They have also described a method for estimating the hydration of α-lactose and also α-lactose in whey solids based on the difference n rates of dehydration (1948). The preparation of pure lactose from whey has been described by Stringer (1939). To crude sugar in 30 per cent solution a decolourising paste (bone black and Norite) is added and the liquid boiled, stood overnight and the acidity adjusted to 0·09 per cent as lactic acid. After standing the acidity is reduced to 0·05 per cent with lime, the liquid boiled and allowed to settle. After twice filtering the liquid is acidified and evaporated. After crystallising the crystals are centrifuged and washed.

Table 2.11—Lactose content of cows' milk (*Overman et al.*)

Breed	*Average per cent*	*Extremes per cent*
Ayrshire	4·69	2·41 to 6·11
Guernsey	4·91	3·57 ,, 5·78
Holstein	4·86	3·96 ,, 5·71
Jersey	4·94	2·73 ,, 5·66
Guernsey-Holstein	4·86	2·98 ,, 6·05

A useful review of the factors controlling crystallisation of lactose in dairy products has been published by Decker and Reid (1944). Mohr and Eysank (1940) have described polarising microscope studies on new crystalline forms of lactose. Stewart *et al.* (1944) conclude that the mode of combination between lactose and casein (e.g. as in spray powder) is obscure, and is not a simple amino-aldehyde condensation or a simple absorption. Three types of reducing groups can be produced by heating a mixture of casein and lactose.

Methods for the manufacture of crude and technical lactose from whey have been described by Webb and Ramsdell (1944), and a review of the utilisation of lactose has been published by Whittier (1944). Sharp and Doob (1941) have given data for the relationship between humidity, moisture content and lactose form in dried whey.

Figures for lactose–ash ratios have been given by Raffaelli (1940) as follows—

	Range	*Mean*
Cow	5·95–7·20	6·57
Buffalo	5·60–9·20	6·86
Goat	5·50–6·82	6·01
Sheep	4·20–6·30	5·00

Anantakrishnan and Herrington (1948) have been able to detect only glucose (besides lactose) in milk. From 4 to 7·5 mg per 100 ml were found in milk and up to 15 mg in colostrum.

Larsen and Gould (1951) have noted that, although picric acid, copper reduction and polarimetric methods for determining lactose in raw milk give virtually identical results, when the milk is heated to high temperatures for a long time the picric acid method indicates a slightly greater loss than the copper reduction method but a considerably greater loss than that given by the polarimetric method.

Babab and Grunpeter (1951) have adapted the Somogyi-Schaffer micro-method for the determination of lactose in dairy products, and Potter (1950) has used the production of blue colour by sugar in the presence of ammonium molybdate and potassium dihydrogen phosphate on heating to follow the hydrolysis of lactose in milk.

McGlasson and Boyd (1951) have studied the recovery of lactose from whey by ion-exchange resins. They found that a lactose of a higher purity could be obtained when whey was not treated with the resin, the average purity being 97 per cent. Tests for the presence of monosaccharides gave negative results.

(3) MINERAL MATTER

On heating milk to 500° C a white ash is left; this contains the mineral constituents of milk, altered, however, to some extent by the oxidation of some of the compounds.

The average composition of the ash of milk is given by Richmond as follows—

2.12—Composition of the ash of cows' milk (*Richmond*)

	Per cent
Lime, CaO	20·27
Magnesia, MgO	2·80
Potash, K_2O	28·71
Soda, Na_2O	6·67
Phosphoric acid, P_2O_5	29·33
Chloride	14·00
Carbon dioxide	0·97
Ferric oxide, etc	0·40
	103·15
Less O as Cl	3·15
	100·00

Other observers have given similar computations; the extremes suggested are given in the following table—

Table 2.13—Composition of the ash of cows' milk (*various observers*)

Constituent	Extremes per cent wt./wt. of ash	Mean per cent wt./wt. of ash
P_2O_5	21·57 to 29·33	25·67
CaO	20·01 ,, 27·32	22·37
MgO	2·25 ,, 3·12	2·63
Cl	13·57 ,, 16·38	14·44
SO_3	Trace ,, 4·11	3·39
Fe_2O_3	0·05 ,, 0·40	0·20
Na_2O	5·82 ,, 11·92	9·03
K_2O	23·63 ,, 30·33	26·34
CO_2	0·00 ,, 0·97	0·97

The amount of insoluble ash, i.e. ash insoluble in hot water, amounts to about 0·52 per cent of the milk; and the soluble ash to 0·23 per cent. The soluble ash consists mainly of the chlorides of the alkalis, with a little carbonate and a mere trace of phosphates.

The insoluble ash is mainly composed of double phosphates of the formula $CaKPO_4$, the lime being partially replaced by magnesia and the potash by soda; double carbonates of the formula $CaNa_2(CO_3)_2$ also exist in traces; these compounds are insoluble in water, and this accounts for the fact that the insoluble ash is always higher than the sum of the calcium and magnesium phosphates.

An ash of this composition is only formed when the milk is homogeneous; if it is curdled by natural souring or by the addition of acids, the precipitated lumps do not contain sufficient alkali metals to form these compounds, and much calcium and magnesium phosphates are formed; on dissolving in water, soluble alkaline phosphates go into solution, and calcium and magnesium phosphates, together with varying proportions of double phosphates, are left insoluble. Curdled milk gives the same total proportion of ash as fresh milk, but the soluble ash is higher and the insoluble ash lower.

Phosphoric acid equal to about 8 per cent of the ash is derived from the phosphorus of the casein; the traces of carbonic acid present are not true mineral constituents of the milk. Deducting these, we have a considerable excess of bases over acids; in the milk these bases are combined partly with the proteins to form soluble salts, and partly with citric acid to form citrates.

Citric acid is contained in milk to the extent of 0·15 to 0·2 per cent; its most characteristic salt is calcium citrate, which is fairly soluble in cold water, but insoluble in boiling water. It is a tribasic acid, and forms three classes of salts.

Sherwood and Hammer (1926) found the citric acid content to vary from 0·07 to 0·33 per cent with an average of 0·18 per cent. Arup (1938) found an average of 0·168 per cent with extremes of 0·150 to 0·206 per cent.

The amount of total ash is of the order of 0·75 per cent. The extremes recorded are 0·47 per cent to 0·99 per cent, but some of the published figures are almost certainly too low. The determination of the ash of milk is a delicate matter, requiring considerable care. The last particles of carbon are not always easy to remove (except by the use of water as described on p. 323), whilst on the other hand the alkali chlorides present are easily volatilised, so that the temperature of ignition should not exceed about 500°. Golding *et al.* (1932) found an average of 0·77 per cent, which is probably very near to the truth; the figure of 0·70 per cent given by Tocher appears to be too low. Cranfield *et al.* (1927) found for 673 samples an average of 0·76 per cent, with extremes of 0·63 per cent to 0·87 per cent; Cornalba (1934) found an average of 0·80 per cent for Lombardy milk.

Soldner deduced the following composition (Table 2.14, p. 36) as most probable for the salts existing in milk.

Other similar computations have been made by van Slyke and Bosworth (1915) and by Porcher and Chevalier (1923), but it is doubtful whether any of these are correct or whether, even if they are correct, they serve any useful purpose.

The percentage of calcium in the original milk, which exists partly in suspension and partly in true solution, has been found by G. van Slyke and Bosworth to vary from 0·126 to 0·217, whilst Burr and Witt (1935) find from 0·120 to 0·204 per cent of calcium oxide, the most common values being 0·140 to 0·159.

Table 2.14

	Per cent
Sodium chloride, NaCl	10·62
Potassium chloride, KCl	9·16
Mono-potassium phosphate, KH_2PO_4	12·77
Di-potassium phosphate, K_2HPO_4	9·22
Potassium citrate, $K_3(C_6H_5O_7)$	5·47
Di-magnesium phosphate $MgHPO_4$	3·71
Magnesium citrate, $Mg_3(C_6H_5O_7)_2$	4·05
Di-calcium phosphate, $CaHPO_4$	7·42
Tri-calcium phosphate, $Ca_3(PO_4)_2$	8·90
Calcium citrate, $Ca_3(C_6H_5O_7)_2$	23·55
Lime combined with protein	5·13
	100·00

Burr and Witt found the percentage of phosphoric acid to vary from 0·168 to 0·275, the most common values being 0·190 to 0·199. Van Slyke and Bosworth found the total magnesium to vary from 0·014 to 0·024 per cent. Table 2.15 gives the extremes and means for a number of the inorganic constituents of milk—

Table 2.15—The inorganic constituents of milk per cent wt/wt

Constituent	Average	Usual variation	Extremes
CaO	0·190	0·175 to 0·190	0·104 to 0·291
P_2O_5	0·220	0·210 ,, 0·255	0·146 ,, 0·310
MgO	0·020	0·015 ,, 0·025	0·005 ,, 0·028
Cl	0·106	0·095 ,, 0·110	0·054 ,, 0·242
Na_2O	0·056	0·046 ,, 0·065	0·036 ,, 0·090
K_2O	0·173	0·160 ,, 0·180	0·148 ,, 0·223

Pyne and Ryan (1950) have studied the colloidal phosphate of milk using the potassium oxalate technique. They find that by this method about 88 per cent of the colloidal phosphate in milk is Ca_3, the remainder probably being Ca_2.

Phosphorus

Basu and Mukherjee (1944) studied the phosphorus distribution in the milks of cows, sheep, goats, buffaloes, and humans, under the headings of inorganic, casein, lipoid and ester phosphorus. The last appears to be mainly creatine phosphate, and human milk contained about half its phosphorus as ester.

Sulphur

Hutchinson (1942a) has found the sulphur content of bulk milk to vary from 0·029 to 0·034 per cent. About 74 per cent of this is lost in ashing. The sulphur in milk may be calculated from the casein, albumin and globulin, or by the equation:

$$S = (\text{ash per cent} \times 0{\cdot}09) - 0{\cdot}34$$

Analytical data for inorganic and organic sulphur in the milk of various mammals have been given by Buruiana and Nicolae (1941).

Masters and McCance (1939) have found the sulphur content of dairy products to be as follows (mg per cent)—

> milk 29·2, cream 33, evaporated 75, sweetened condensed 82·5, skim condensed 94·3, ice cream 30·6, butter 9·1, Cheddar 230, Dutch 186·5, Gruyère 206, Gorgonzola 177, Stilton 228, Parmesan 251, processed 321 and St. Ivel cheese 186.

Diemair *et al.* (1939) report values for volatile sulphur as follows (mg per 100 ml milk): raw 0·13–0·15, holder pasteurised 0·11, high-temperature pasteurised 0·11–0·14, roller whole 0·13, roller skim 0·19, Krause spray whole 0·22, Krause spray skim powder 0·27. Other products mg per 100 g: cream 0·07–0·11, cream cheese 0·37–0·55, Camembert cheese 0·15 and butter 0·2.

Townley and Gould (1943) have studied the origin and factors controlling the liberation of the volatile sulphides of milk. Milk, etc., heated to 90° C gave off the following amounts of volatile sulphide (as mg) per litre: milk 0·24, skim milk 0·16, cream 0·48, buttermilk 0·51, skim milk whey 0·21, buttermilk whey 0·58. The sulphydryl compounds appear to come from both milk serum and the fat globule membrane, the latter liberating the sulphur compounds more easily. The production from whey was at a maximum at pH 9, and oxidising agents tended to decrease and reducing substances usually increased it.

Trace metals

Values for heavy-metals contents of dairy products are given in Table 2.16 (Schwaibold and Lesmüller 1948).

Table 2.16—Trace metal contents of dairy products (p.p.m.)

	Butter	Whole milk	Casein	Milk protein	Milk powder	Cheese (various types)
Copper . .	0·2–1·4	0·2–0·3	3·8	3·4	2·4–2·5	0·4–2·3
Lead . .	0	0	1·5	2·5	1·2–2·5	0
Zinc . .	0·8–2·6	0·6–2·4	3·7	28	13–28	2·3–6·7

Kehoe *et al.* (1940) report values of 0·02–0·04 p.p.m. for lead in milk. Plumier (1947) found human milk to contain 58 μg (25–121) iron per cent and cows' milk 45 μg per cent. Pal (1940) has obtained values of 0·635 and 0·667 mg per cent[1] for ionisable and total iron in cows' milk. The corresponding values for human milk were 0·625 and 0·633. Ahmad and McCollum (1939) have reported values of about 0·006 mg per cent dry matter for cobalt in milk powders. Archibald (1949) has made the interesting observation that, even if fed nickel salts, cows do not pass the metal into their milk. Nickel thus behaves quite differently from cobalt, a constituent of vitamin B_{12}. Values for zinc (Archibald, 1944), cobalt (Archibald, 1947), manganese (Archibald and Lindquist, 1943) and molybdenum (Archibald, 1951) have also been published in America. Rudra (1940) has found values of 0·006 and 0·0075 mg per cent for manganese in cows' and goats' milk. Allport and Garratt (1948) have given a critical account of methods for estimating metallic elements in foods, and Forstner (1948) gives values for a number of foodstuffs. Nutritive aspects have been reviewed by Cuthbertson (1948).

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[1] Query: per 1000 ml.—*J.G.D.*

According to Itzerott (1943) the initial copper content of milk is 0·05–0·14 (mean 0·07) and of iron 0·3–0·77 (mean 0·5) p.p.m. Commercial handling increased these values, and the maximum contents of Cu and Fe which do not induce oiliness, etc., are for milk 0·2 and 1·5–2·0, for butter 0·2 and 2, for condensed milk and milk powder 2·5–3·0 and 5 to 6. The metals are distributive according to the protein in separation, and in churning adhere to the fat globule membrane presumably as a metal–protein complex. Wertz *et al.* (1949) report values for iron in milk of 0·25–0·38 (mean 0·3) mg per litre.

Bibliographies on metals in foods and biological materials have been published as follows—

I Cobalt (1940, *Analyst*, **65**, 513)
II Nickel (1940, *Analyst*, **65**, 603)
III Manganese (1941, *Analyst*, **66**, 196)
IV Zinc (1941, *Analyst*, **66**, 452)
V Copper (1942, *Analyst*, **67**, 293, 324 and 357)
VI Bismuth (1943, *Analyst*, **68**, 115 and 217)
VII Cadmium (1944, *Analyst*, **69**, 51)

Values for trace elements in foods have also been given by Holland and Ritchie (1943) and by Monier-Williams (1949). The toxic effect of metallic contaminants in foods is discussed by Monier-Williams (1948).

Trace elements (non-metals)

Evans and Phillips (1939) report a value of 0·2 p.p.m. fluorine in milk, of which 25 per cent was in the protein. Ford *et al.* (1940) have reported values for bromine in milk from 1·2 to 2·6 p.p.m. Hove *et al.* (1939) have obtained values for boron of 100–200 μg per litre.

Chemical stability

The chemical stability of fresh milk (excluding colostrum) is controlled chiefly by the balance of calcium and magnesium to phosphate and citrate ions. When fresh milk is unusually prone to clot on boiling, this tendency may be corrected by the addition of sodium citrate (Seekles and Smeets 1947). No correlation could be found with soil or season, but extremes of feeding may be a contributing factor. In practice there is a period of instability extending over about two weeks in April–May, which is recognised in the industry and is of special concern to condensing creameries. The alcohol test is the most useful for detecting those milks likely to give trouble in condensing through physical instability. At such periods even bulk pasteurised milk may deposit gelatinous floccules on the side of a glass container if the milk is heated in it for some time.

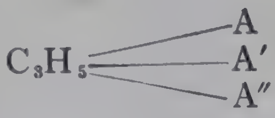
Colostrum is probably a major factor in this seasonal instability as the autumn calving campaign has made it necessary for condensing creameries to take special precautions to keep out such milk in autumn as well as in the spring.

(4) FAT

The variations in the amount of fat found in different milks will be dealt with in Chapter III. This section deals with the composition of butter fat and the variations likely to be found.

The milk fats of some mammals differ very sharply from most other fats in that they contain notable quantities of butyric acid. Cow-milk fat contains about 4 per cent of butyric acid, whilst those of the buffalo, sheep and goat contain somewhat similar amounts, the buffalo having a little more and the sheep and the goat a little less. Milk fats containing smaller but appreciable amounts of butyric acid are those of the ass, rabbit and camel. Other milk fats contain much smaller amounts, details of which will be found under the milks in question. In this section only cow-milk fat will be dealt with.

This, like all other fats, consists of a mixture of glycerides, i.e. esters of glycerol. It is now well known that the presence of simple triglycerides in a fat is the exception rather than the rule, and that they are only present when the formation of mixed triglycerides is impossible owing to the presence of a predominating portion of one acid. The normal constitution of a fatty glyceride may therefore be written as—



$$C_3H_5\begin{cases}A\\A'\\A''\end{cases}$$

where A, A′, and A″ are different fatty acids.

That butter fat contains no glycerol tributyrate is shown by the following facts—

(1) It would be possible to dissolve out the glycerol tributyrate with alcohol; which cannot be done.

(2) It would be possible to distil off glycerol tributyrate under reduced pressure; this cannot be done.

Several definite mixed glycerides have been isolated from butter fat.

The first serious quantitative examinations of butter fat were made by Duclaux (1886) and somewhat later by Violette (1890). These results, together with others due to Bell, Blyth and Spallanzani, are now only of historical interest. The more important of the later results are those due to Crowther and Hynd (1917) and to Holland and Buckley (1918), who used an extended modification of the method of alcoholysis orginally devised by Haller (1906). These results, although a considerable advance over anything previously attempted, are not now regarded as of sufficient accuracy, as the necessity for prior separation of the saturated and unsaturated higher fatty acids by means of their lead salts was not then appreciated.

One of the first computations of the proportion of the fatty acids contained in butter fat which has serious claims to accuracy is that due to Hilditch and

Jones (1929), who examined three bulk samples of ordinary deliveries of New Zealand butter by the methods developed by Hilditch and his co-workers (1927), (1928a), (1928b). The following results were obtained—

Table 2.17—Proportions of individual fatty acids in milk
(Hilditch and Jones)

Acid	Sample (per cent)		
	A	B	C
Butyric	3·4	3·1	3·2
Caproic	1·8	1·9	1·7
Caprylic	0·9	0·8	0·8
Capric	1·9	2·0	2·3
Lauric	3·1	3·9	4·3
Myristic	9·7	10·6	10·8
Palmitic	27·6	28·1	28·4
Stearic	12·2	8·5	9·4
Arachidic	0·7	1·0	0·5
Oleic	34·3	36·4	33·1
Linoleic	4·4	3·7	5·4

Hilditch has since stated (1947) that the proportions of oleic acid are probably 3 to 5 per cent too high, since they include unsaturated acids of lower molecular weight.

Hilditch and Sleightholme (1930) found that the influence of fat added to the feed is definite, although small, and there are much smaller alterations due to other factors such as the main character of the feed and the climate. The differences found relate mainly to variation in the amount and nature of the unsaturated acids present, and also to some extent in the amount of lower saturated acids. These results, together with others obtained by Hilditch and Thompson (1936), show that the feeding of oils rich in linoleic acid does not produce any significant increase in the amount of polyethenoid C_{18} acids. The feeding of rape oil produced a notable proportion of erucic acid, whilst the feeding of cod liver oil caused a very considerable reduction in the amount of lower saturated acids, with a proportional increase in oleic acid. There was also a notable increase in the C_{20} and C_{22} unsaturated acids, but no increase in the amount of hexadecenoic acid, in spite of the considerable amount of this latter acid which is present in cod liver oil. The results obtained are set out in Table 2.18.

In some of the following analyses, as in many of the later ones, some or all of the minor unsaturated compounds are separately included. The decenoic acid is 9 : 10 $C_{10}H_{18}O_2$; the tetradecenoic acid is 5 : 6 $C_{14}H_{26}O_2$; the hexadecenoic acid is 9 : 10 $C_{16}H_{30}O_2$, commonly known as palmitoleic acid; the octadecadienoic acid is 9 : 10, 12 : 13 $C_{18}H_{32}O_2$, commonly known as linoleic acid.

Table 2.18—Effects of oils and fats in feed on composition of butter fat

Acid, per cent wt./wt.	Cows fed on—					
	Coconut cake	Soya-bean cake	Linseed oil	Rape oil	Cod Liver oil (1)	Cod Liver oil (2)
Butyric	3·4	3·6	4·2	3·6	2·1	2·0
Caproic	2·0	1·5	2·0	1·6	0·9	0·6
Caprylic	1·1	1·7	1·3	1·0	0·5	0·6
Capric	3·2	3·8	2·3	1·5	1·2	1·3
Lauric	7·3	6·5	3·1	1·8	3·1	0·9
Myristic	17·1	10·6	8·4	8·1	6·4	8·4
Palmitic	27·0	26·3	21·8	20·3	22·7	25·4
Stearic	4·8	8·3	9·9	13·8	6·7	8·2
as Arachidic	—	1·2	0·6	0·5	0·6	0·6
,, Decenoic	—	—	—	0·2	—	—
,, Tetradecenoic	—	—	—	1·3	—	—
,, Hexadecenoic	—	—	—	2·4	—	3·3
,, Oleic	31·7	32·9	39·3	36·0	43·3	37·8
,, Octadecadienoic	2·4	3·6	5·9	3·3	4·8	3·9
,, C_{20-22} unsaturated	—	—	1·2	1·0	—	—
,, Erucic	—	—	—	3·6	—	—

A number of other analyses have been made by Hilditch and his collaborators and by other workers. A representative selection of the figures obtained is given in the following table—

Table 2.19—Composition of butter fat

Acid, per cent wt./wt.	English stall-fed		Cow no. 1‡—1938		Cow no. 2‡—1938
	1935*	1937†	Before fasting	During fasting	During fasting
Butyric	3·7	3·0	3·5	1·2	2·7
Caproic	2·0	1·4	0·6	—	0·1
Caprylic	1·0	1·5	1·0	0·1	0·1
Capric	2·6	2·7	1·8	0·2	1·0
Lauric	1·7	3·7	2·5	0·1	0·6
Myristic	9·3	12·1	11·9	2·8	3·8
Palmitic	25·4	25·3	23·5	20·0	22·1
Stearic	10·7	9·2	11·6	14·3	9·9
as Arachidic	0·4	1·3	1·1	0·9	0·9
,, Decenoic	0·2	0·3	0·2	—	0·2
,, Dodecenoic	—	0·4	0·2	—	0·2
,, Tetradecenoic	1·2	1·6	0·9	0·4	0·2
,, Hexadecenoic	5·0	4·0	3·2	1·4	1·2
,, Oleic	32·4	29·6	35·9	52·8	51·7
,, Octadecadienoic	4·0	3·6	1·2	2·5	0·8
,, C_{20-22} unsaturated	0·4	0·3	0·8	3·3	3·5

* Hilditch and Paul (1936). † Hilditch and Longenecker (1938).

‡ Smith and Dastur (1938).

Hilditch (1937) has summed up the knowledge of the minor constituents of butter fat as far as the facts available will allow. He states that (taking molar percentage figures as a true indication of composition) oleic, followed closely by palmitic, is the predominating fatty acid, whilst butyric, myristic and stearic come next in amount with about equal proportions. As a first approximation, the following may be regarded as the molar composition of butter fat—

Table 2.20—Molar composition of butter fat

Acid	*Per cent (molar) present*
Butyric	8
Caproic	3
Caprylic	2
Capric	3
Lauric	4
Myristic	9
Palmitic	25
Stearic	8
Oleic	33
Other unsaturated and undetermined	5
	100

The amount of arachidic acid present is somewhat doubtful. The figure for this acid usually given probably includes saturated acids up to those containing C_{26}. In earlier analyses, figures were usually given for the amount of linoleic acid, $CH_3(CH_2)_4CH{=}CH\,.\,CH_2CH{=}CH(CH_2)_7\,.\,COOH$, but it is extremely doubtful whether this acid is present, at any rate to any material extent. The octadecadienoic acids present appear to be geometrical isomerides of linoleic acid. Traces of more highly unsaturated acids are also present. Brown considers these to be exclusively arachidonic acid, $C_{20}H_{32}O_2$. Hilditch is of the opinion that whilst this acid may be the chief component, there are probably other polyethenoid acids present, possibly of the C_{22} or the C_{20} series.

The more recent analyses appear to show that the usual variations likely to be found are those given in Table 2.21. It should be emphasised, however, that a very large proportion of market butter, prepared as it is under

Table 2.21—Variations in the composition of butter fat

Acid	*Per cent present*
Butyric	3·0 to 4·5
Caproic	1·3 ,, 2·2
Caprylic	0·8 ,, 2·5
Capric	1·8 ,, 3·8
Lauric	2·0 ,, 5·0
Myristic	7·0 ,, 11·0
Palmitic	25·0 ,, 29·0
Stearic	7·0 ,, 13·0
Oleic	30·0 ,, 40·0
Other components	3·0 ,, 6·0

more or less standardised conditions with regard to the food and environment of the cattle, is not likely to show such wide variations. As far as the volatile acids and unsaturated acids are concerned, the statistics given in Chapter X will indicate the more usual variations, although both higher and lower results have been recorded. The effect of unusual foods and environment is dealt with below.

For further details of the latest work on the chemistry of milk fat the reader is referred to Hilditch (1947).

Influence of feed on composition of butter fat

Many workers have produced evidence to show that whilst changes in feed have little effect on the percentage of fat present in milk, they may have a considerable effect on the composition of the fat. Most of this work has dealt with the effect of feeding on the usual analytical constants, such as iodine value and Reichert value—these are dealt with in a later chapter.

Hilditch and Sleightholme (1930), (1931) showed that the influence of added fat in the diet, although definite, is of a minor order compared with that due to other causes. More profound changes are brought about by such influences as the change from outdoor to indoor life, the general character of the diet, and seasonal changes in the temperature. These changes lie mainly in the variation of the amount of unsaturated acids present, and in the amount of the lower saturated acids present. Palmitic acid appears relatively to be the most constant of any of the components; the amount of stearic acid found varies considerably. When coconut oil formed part of the diet, the fully saturated part of the butter fat showed a marked increase in butyric–lauric acid content, but for the most part the composition of the non-fully-saturated glycerides was normal. With soya-bean oil forming part of the diet, the fully-saturated glycerides were not far removed from the normal in composition, but the lower acids of the non-fully-saturated portion were present in excess of the usual proportion.

Dean and Hilditch (1933) have shown that an abrupt increase in the proportion of unsaturated acids, with a corresponding diminution in butyric and stearic acids, occurs in the spring, when cows are put out to grass. They also show that there is a gradual increase in the unsaturated acids as the age of the animal increases, largely at the expense of the palmitic acid.

Hilditch and Thompson (1936) have studied the effect of additions of cod liver, linseed, or rape oils to the diet. Cod liver oil reduced the lower saturated acids to about half the normal content, the amount of oleic acid was much in-increased, and a notable amount (5 to 7 per cent) of highly-unsaturated C_{20-22} acids was present. Somewhat strangely, it was found that polyethenoid unsaturation in the C_{18} acids was not more than normal, and that palmitoleic acid had not been appreciably absorbed from the cod liver oil. The effect of linseed oil was mainly to increase the proportion of oleic acid; linoleic acid was not detected and linolenic acid only in very small amounts. Rape oil had a similar effect to linseed oil, and in addition small amounts of erucic acid were present.

Hilditch and Jasperson (1941) have studied the effect of pasture and silage on the acid constitution of the milk fat. Pasture reduced and silage increased the butyric acid. Silage increased the palmitic and hexadecenoic, and decreased the stearic and oleic acid. Silage increased the C_4 to C_{14} acids by 12 per cent, and the C_{16} by 15 per cent, but decreased the stearic by 15 per cent and the oleic by 18 per cent compared with the pasture butters. Brouwer and Jonker-Scheffner (1947) have reported that grass feeding increases the proportion of vaccenic acid (a solid isomer of oleic acid). According to Anantakrishnan *et al.* (1948), cotton seed, sesame and hydrogenated peanut oil in the diet of cows produce an increase in the iodine number and a decrease in the Polenske value of the butter fat.

Hilditch and Meara (1944), have shown that in human milk fat there are roughly equal amounts of saturated and unsaturated fatty acids. The former consist chiefly of palmitic with stearic and myristic; the latter are mainly oleic, with some hexadecenoic and tetradecenoic and octadecadienoic (chiefly linoleic) acids. Human milk fat therefore differs from bovine in the higher content of unsaturated and the absence of acids below C_{10}. Human milk fat thus resembles margarine fat.

Parker *et al.* (1951) have recently studied factors affecting the degree of unsaturation of milk fat. They have found that pasture and stage of lactation have marked effects whereas changes in temperature have little effect.

Grazing increased the iodine value about 5 units and this increase was sustained throughout the period of grazing. Pasture feeding, however, had little effect on the linoleic acid content, but changes in the iodine value appear to be chiefly associated with the oleic acid content of the fat.

Generally a high iodine value was found at the peak of milk production. This was followed by a decline of about 8 units in iodine value up to the fourth or fifth month of lactation. A slight increase, about 3 iodine units, then took place up to the end of lactation.

Fredeen *et al.* (1951) have shown that the susceptibility of single cow samples to spontaneous rancidity varies according to the cow, stage of lactation and season. Advanced lactation as such and not advanced gestation appears to be the factor controlling the increased tendency to rancidity which occurs towards the end of lactation. There appears to be no relation between the oestrus cycle and rancidity susceptibility.

Glycerides of butter fat

The amount of work which has been done on the glyceride structure of butter fat is relatively small and has been almost entirely contributed by Hilditch and his collaborators. This work was reviewed and extended in 1940. It has already been stated that simple triglycerides are not found in butter fat to any extent. Amberger (1913) (1918) tried separation of individual triglycerides by fractional crystallisation. Only small quantities of pure substances were isolated, which included palmito-distearin, dipalmito-stearin, oleo-dipalmitin and oleo-butyro-palmitin. Arup (1928) separated a sample of Irish butter fat into several fractions by keeping it for a time at each of several progressively diminishing temperatures. The analytical characteristics of the various fractions suggested that the acids of low molecular weight and also the higher-saturated acids are distributed more or less evenly throughout the glycerides. The work of Hilditch has shown that the component acids of the considerable amounts of fully-saturated glycerides present in milk fats were not widely dissimilar in their relative proportions to those saturated acids present in combination as mixed saturated-unsaturated glycerides. Results from a number of butter fats which varied in oleic acid content over a fairly wide range show that the proportion of fully-saturated glycerides present varies inversely with the content of oleic acid, and that while the proportion of palmitic acid tends to constancy and that of stearic acid is relatively low (although variable), the characteristic lower-saturated acids of the milk fats also vary inversely with the proportion of oleic acid, the sum of these two groups tending to be approximately constant in amount. Hilditch has resolved butter fat into three fractions by crystallisation from acetone, and has subjected each fraction to a detailed examination. The large number of fatty acids present in butter fat rendered impossible anything in the nature of an exact description of the many mixed glycerides which are likely to be present, but the general distribution of the four main groups of acids—oleic, palmitic, stearic, and the lower acids of the C_4 to C_{14} series—was approximately obtained. The mixed glycerides were found to be present to approximately the following extents in molar percentages: oleo-C_{4-14}-palmitins, 31 to 22; oleo-palmito-stearins, 8 to 17; palmito-dioleins, 17 to 4; oleo-C_{4-14}-stearins, 12 to 6; smaller proportions of oleo di-C_{4-14}, 4 to 9; C_{4-14}-dioleins, nil to 10; stearo-dioleins, 8 to 1; oleo-dipalmitins, 1 to 5; trioleins, nil to 7; fully-saturated glycerides (C_{4-14}-palmito-stearins, 9; di-C_{4-14}-palmitins, 6 to 7; and small amounts of di-C_{4-14}-stearins, C_{4-14}-dipalmitins, dipalmito-stearins, and palmito-distearins) amounting in all to 19 per cent. The most abundant components are glycerides containing one radical each of oleic, palmitic, and one of the lower acids (from C_4 to C_{14})—22 to 30 per cent of the whole fat; nearly 40 per cent of the fat is made up of the four groups oleo-C_{4-14}-palmitins, oleo-palmito-stearins, palmito-dioleins and oleo-C_{4-14}-stearins. There is also 19 per cent of fully-saturated glycerides. No evidence of the presence of tributyrin or other simple triglyceride was found, but a small proportion of glycerides was obtained which were composed entirely of unsaturated acids.

Arup (1928) examined a number of Irish butter fats which he divided into different fractions by removing the liquid portion, which separated at temperatures varying from 10° to 37° C, and then allowing some of the fractions to crystallise out at a lower temperature. The analytical results given in Table 2.22 were obtained on examination of the respective fractions from three separate butter fats.

As Arup points out, the figures cannot be interpreted as indicating any marked tendency for the volatile acid groups to be associated with the oleic acid groups rather than with the stearic and palmitic acid groups, but on the whole they support the theory of impartial mutual distribution of the acid groups among the different glycerides.

Detailed analyses of the constituent fatty acids of milk fat have been given by Hilditch *et al.* (1940).

A spectro-photometric method for the determination of linoleic acid in milk fat has been described by Schaffer and Holm (1950). They have found values of 2·1 to 2·7 per cent for linoleic acid and values of 0·8 up to 1·3 for octadecatrienoic acid calculated as linolenic acid.

Butter fat has been reported to contain 0·5 to 0·7 per cent of vaccenic acid (Geyer *et al.* 1947). For details of fatty acids and fat constitution the reader is referred to Bloor (1943), and Hilditch (1947).

Sharp and Krukovsky (1939a) have studied the solidification of milk fat and point out that fat in tiny globules crystallises more slowly than in a mass as any seeding crystals can only affect that particular globule. At 20–25° C little crystallisation takes place, and even at 15° C it requires four hours. A marked decrease in volume occurs during this time, and this is followed by a very slow expansion. Thiel (1944) found that butter fat can dissolve 0·19 per cent water at 40°, 0·26 at 60°, 0·36 at 80° and 0·47 per cent at 95° C. Cholesterol, lecithin and salts had no effect on solubility.

Fitelson (1943), has reported that butter fat contains 7 mg squalene per cent.

A review of existing knowledge on the nutritive value of butter fat, margarine and other fats has been given by J. A. B. Smith (1948).

Table 2.22—Fractional analysis of butter fats

	Reichert* value	Polenske value	Kirschner value	Iodine value
Original butter fats ..	30·4	1·9	22·6	39·2
	30·9	2·2	22·4	37·3
	30·9	2·6	21·6	38·5
Portions liquid at 10° C ..	33·4	2·6	25·1	48·9
	35·5	3·0	26·3	47·5
	34·8	2·9	24·9	46·5
Portions liquid at 15° C ..	32·9	2·5	25·0	41·0
	34·5	2·9	25·2	40·8
	34·7	3·0	24·4	40·5
Portions crystallised at 15° C	32·2	2·3	23·8	37·9
	33·4	2·5	24·5	26·3
	30·5	2·8	22·8	38·5
Portions crystallised at 20° C	29·0	2·2	23·6	35·1
	30·4	2·3	22·4	35·6
	29·6	2·8	20·1	36·8
Portions crystallised at 27° C	27·2	2·2	19·8	33·1
	27·9	2·2	19·1	34·0
	28·6	2·6	19·1	24·2
Portions crystallised at 37° C	21·5	1·9	16·5	26·7
	25·0	2·2	18·4	30·7
	25·3	2·3	16·3	23·2

* These methods are explained in Chapter 10, and described fully in Chapter 23.

Oxidation of milk fat

Keeney and Doan (1951) have obtained material with the characteristic odour of oxidised milk fat by vacuum distillation. Carbonyl compounds were found to be present, and the addition of the volatile neutral material to ordinary milk in concentrations of 1 p.p.m. was found to impart the characteristic flavour.

The preparation of 2,4-dinitrophenylhydrazones from this material indicated that carbonyl compounds of the following empirical formulae were present: $C_8H_{16}O_2$, $C_7H_{12}O$, $C_9H_{16}O$, $C_{12}H_{20-22}O$. None of these ketones was found to be a methyl ketone and most of them appear to be unsaturated. It is possible that lactones are also present in the volatile material, and these were correlated with the fruity and coconut odour of certain fractions of the distillate. The oxidised flavour could be closely simulated by the addition of suitable proportions of the ketone fraction and the non-carbonyl neutral fraction.

These workers suggest that the changes in flavour with the development of the taint are due to different proportions of the flavour compounds.

Patton and Kurtz (1951) have shown that spectrographic analysis of the red colour developed when oxidised milk fat is heated with 2-thiobarbituric acid (TBA) is identical with the colour produced when TBA is heated with malonic dialdehyde. They suggest that malonic dialdehyde may be a compound of significance in biological oxidation of unsaturated fatty acids and in the development of rancidity in food. The TBA reagent has been found to be more sensitive than tests usually employed, such as the Kreis test and the iodine value, and the authors describe a procedure for the empirical measurement of fat oxidation. They suggest that the TBA test can be used for the measurement of oxidative deterioration in many kinds of fats and fat-containing foods.

Phosphatides in milk

Heinemann (1939) gives the following values for " lecithin ": raw milk 0·035, skim 0·15–0·18, skim from re-separated cream 0·035–0·093, pasteurised cream 0·006–0·199, buttermilk 0·114–0·126, butter 0·153–0·212 and separator slime 0·229 per cent. By using an adsorption-column method Horrall and Crane (1942) found from 0·2 to 0·5 per cent phosphatides in butter fat.

Hilditch and Maddison (1941) give the following figures for acids of phosphatides isolated from Swiss and English butter fats: myristic 3·2, 5·5; palmitic 21·0, 13·4; stearic 7·3, 9·0; " arachidic " 12·3, 20·9; oleic 32·2, 23·5; hexadecenoic 4·3, 4·9; octadecadienoic 6·4, 0·0. Thus the lower fatty acids of butter fat are absent from the phosphatides. Lecithin has been found present in the fat of milk products in the following concentrations: whole milk 0·18 to 0·49, colostrum 0·57, cream 0·09 to 0·26, skim milk 1·34 to 3·9 and evaporated milk 0·11 to 0·43.

Kabane and Levy (1945) have published a comprehensive paper on choline. They found average values of 15 for free choline and 50 for soluble choline mg per litre. Engel (1943) found the choline content of fresh milk, whole milk powder, skim milk powder and Cheddar cheese to be 1·14, 1·1, 1·63 and 0·7 mg per g dry matter as choline. Butter and casein contained 0·05 mg.

Kurtz and Holm (1939) find that the ether- and acetone- insoluble milk lipoid fraction consists about half of sphingomyelin and half of cerebrosides.

Nataf *et al.* (1948), have reported an average value of 11·3 mg cholesterol per 100 ml for cows' milk, with a range of 7 to 17. They found a good correlation between cholesterol and fat contents in milk, but not between cholesterol in milk and that in the blood serum.

(5) THE MINOR CONSTITUENTS

(A) THE ENZYMES OF MILK

An enzyme is a catalyst (i.e. a substance which greatly increases the velocity of a chemical reaction without itself undergoing any permanent change) formed by a living organism.

The presence in milk of a considerable number of enzymes has been reported. Some of these occur in the milk as drawn, others are introduced as a result of bacterial contamination. A very complete résumé of the work on this subject up to the year 1912 is contained in a Report to the Local Government Board (now the Ministry of Health) by Lane-Claypon (1912) where a fuller account of certain aspects of the subject will be found, particularly with regard to the physiological importance of enzymes.

The enzymes that have been reported as present in milk may be divided into the following classes—

(1) Peroxidase: giving the peroxidase test.
(2) Catalase: decomposing hydrogen peroxide.
(3) Lipolytic enzymes: decomposing fats.
(4) Phosphatase: decomposing phosphoric esters.
(5) Reductases.
(6) Proteolytic enzymes: decomposing proteins.
(7) Lactase: decomposing lactose.
(8) Amylase (diastase): decomposing starch.

The enzymes which may be present in perfectly fresh milk are peroxidase, catalase, reductase, phosphatase, lipase and amylase (the last two in small amounts); the others, when present, are found as a result of bacterial contamination.

Peroxidase

When fresh milk is mixed with tincture of guaiacum and a little hydrogen peroxide, a characteristic blue coloration is produced which is due to the action of an oxidising enzyme. This enzyme is now called " peroxidase ". The reaction is not due to bacterial contamination, and it is not given by milk which has been heated much above 70°, but the time of heating, as well as the temperature, is a factor. For example, according to Bouma and van Dam (1918) the reaction is not given by milk which has been heated to 70° C for 150 minutes, 75° C for 2·5 minutes or 80° C for 2·5 seconds.

The peroxidase reaction is usually made evident by the oxidation of some substance which gives a bright colour on oxidation. Where the peroxide is absent, the test will not operate even in the presence of peroxidase—for this reason hydrogen peroxide is usually added. This is the explanation for the failure of fresh tincture of guaiacum to give the reaction in the absence of hydrogen peroxide, whilst old tinctures (which contain peroxide) give it.

Lactoperoxidase was not only the first enzyme to be detected in milk but was also the first to be crystallised. It is a haemin protein, having the properties of an albumin. The molecular weight is about 93,000.

A useful survey of animal peroxidases has been issued by Polonovski and Jayle (1939) who state that the Arakawa method of testing is 64 times as sensitive as those of Schroeter and Bourquelot.

According to Vorstman (1948) milk only gives a Storch test (peroxidase eaction) at pH values between 5·4 and 8·4, the optimum being 7·0 to 7·5. Krukovsky (1949) has shown that hydrogen peroxide does not oxidise ascorbic cid in milk if the milk is first heated to 76·6° C. Addition of plant peroxidase o the heated milk permits the reaction to proceed. Grimmer and Kleiman 1939), have described the preparation and behaviour of milk peroxidase. Peptone protects and drying destroys the enzyme. Theorell and Åkeson (1947) have prepared a highly purified peroxidase from milk. It is a haemin protein, brownish-green in the ferri- and emerald green in the ferro- form, and is albumin in nature. They find it different from plant peroxidases. Theorell and Pedersen (1947) report a molecular weight of 93,000 and suggest that the molecule contains one atom of iron.

It is well known that peroxidase inactivated by heat can become reactivated later. Fischer (1943) explains this by postulating that the " carrier protein " becomes coagulated and the enzyme is then absorbed on other coagulated milk proteins. The gradual redispersion of the coagulum on standing leads to a recombination, but the reactivated enzyme is more thermo-labile. The destruction of the enzyme follows the course of a monomolecular reaction.

Although peroxidase is present in all cows' milk, it is usually found in greater amounts in the milk of cows suffering from diseased glands; it is, however, found in greatest concentration in the slime obtained from separators. Like most of the enzymes, it is active at about pH 6; should the pH extend, however, beyond the range pH 3·2 to pH 10 it is completely destroyed.

Catalase

This is the enzyme which is capable of decomposing hydrogen peroxide into molecular oxygen and water. It occurs in leucocytes and epithelial cells in milk, and the amount increases rapidly as a result of mastitis. Colostrum and separator slime contain higher amounts of catalase than milk. The preservation of milk with hydrogen peroxide (Budde's process) is due to the destruction of bacteria by oxygen liberated from the peroxide by catalase.

Catalase is precipitated with casein and has a maximum activity at pH 6·8 and 7·0. It is destroyed by heating at 65°–70° C for 30 minutes.

Lipase

The fact that lipolytic activity occurs in milk and other dairy products has been known for some years, but doubt has from time to time been expressed whether this activity was due to an enzyme originally present or whether it was due to micro-organisms which have gained access to the milk. Rice and Markley (1922) found evidence of the presence of a true lipase in raw milk (by " true lipase " is meant an enzyme capable of acting on neutral fat, as distinct from specific butyrinases which are only capable of splitting alkyl butyrates or other simple esters). More recently the presence of a tributyrinase (which is probably a true lipase) has been confirmed by the work of Mattick and Kay (1938). This latter enzyme was found to show great activity in colostrum, the amount decreasing rapidly in the first few days of the lactation period and then remaining fairly steady during the remainder of the period. The enzyme is not apparently associated with the fat of the milk as, on centrifuging, the activity of the separated milk is always considerably greater than that of the cream. It shows its greatest activity at pH 8·2 to 8·7, and it is one of the more thermolabile enzymes of milk, being destroyed to the extent of over 98 per cent by heating at 56° C for 30 minutes; it is therefore distinctly more unstable than phosphatase (see below). The above workers and Krukovsky and Herrington (1938) found considerable variation in the lipase activity in the milk of individual cows. Pasteurisation destroys this enzyme in milk and should always be carried out before the milk is condensed, as the latter process is otherwise favourable to lipolysis. Butter made from sweet cream which has not been pasteurised is also liable to be affected by this enzyme. Other conditions which lower the activity of the enzyme are traces of heavy metals, particularly copper, and high acidity. Homogenisation, on the other hand, increases lipolytic activity, and Gould (1914) has studied the effect of the temperature of homogenisation on the activity, which he found to be greatest at 105° F.

Herrington and Krukovsky (1939) have emphasised the individuality of the cow in respect of lipase-induced increases in fat acidity. The enzyme can be activated by warming to 30° C, and rapid lipolysis is attained by cooling to 15° C. There appeared to be no relation between lipase activity and stage of lactation or milk production. The proportion of formalin-sensitive lipase also appeared to vary. Johnson and Gould (1949) have described an alcohol–ethyl ether–petroleum ether extraction method for following lipase action in milk which gives a higher yield of fatty acids. Adjustment of the milk to pH 2 increased the recovery, but saturation with $MgSO_4$ or NaCl had little effect.

Hetrick and Tracy (1948a) have found the following time–temperature conditions to destroy lipase in milk: 186° F—0·03 sec.; 173° F—1 sec.; 167° F—2 sec.; 160° F—10 sec.; 155° F—22 sec.; 150° F—120 sec.; 145° F—500 sec. At 145° F lipase was destroyed in one-third the time required for phosphatase, but at 185° F the same time was required for both enzymes. Added copper did not influence the rate of heat inactivation of lipase.

Krukovsky and Sharp (1940) have confirmed Davies' finding that lipase can be destroyed in the presence of heavy metals at low oxygen tension. Copper is without effect in the absence of oxygen. Hlynka and Hood (1942) have been able to secure partial reactivation of oxidised lipase in the absence of Cu by de-aeration and by cysteine. The latter also protected the enzyme in aerated milk, but ascorbic acid failed to give consistent results. Kay (1946) found that sunlight can destroy 40 per cent of the tributyrinase. Blue light is most effective and riboflavin increases the loss.

Tarassuk and Richardson (1941a) have shown that there is no splitting of fat in milk in the udder, and that the enzyme is inactive at 20–37° C but becomes activated on cooling to below 20° C. Agitation without cooling can also activate the enzyme, and the authors suggest that permeability of the fat globule membrane is the responsible factor. According to Roahen and Sommer (1940) gravity cream is more easily attacked than separator cream. Cream separated at 110° F showed less lipolysis than that separated at 75° F. The optimum pH is 8·5 and formaldehyde partly inhibits lipolysis. There is no relation between stage of lactation and lipase content. Krukovsky and Sharp (1940a) have further studied the effect of fat properties and surface on lipolytic activity. Lipase attacks more strongly those fat fractions richer in the lower fatty acids and also " resurfaced " fat globules. Gould (1940a) has given the results of experiments on different ways of homogenising milk in relation to subsequent lipolysis. A maximum effect occurred when milk was homogenised at 105°–115° F and much less at 135° F.

According to Lythgoe and Rosenthal (1943) goats' milk contains less lipase than cows'. The " goaty flavour " is considered to be due to the higher proportion of caprylic acid in goats' milk fat.

A comprehensive review of the lipase in dairy products with special reference to faults produced by lipase action and their control has been written by Herrington (1950).

In addition to the above enzymes, which may be termed true lipases, Grimmer (1913) and other workers have established the presence of a monobutyrinase in milk, and Vandevelde (1908) has reported the presence of an enzyme capable of splitting phenyl salicylate (salol).

Phosphatase

Work on the phosphorus compounds of milk has been reviewed by Allen (1931). Demuth (1925), Wilson and Hart (1932) have worked on the phosphatase present in milk. Graham and Kay (1933) have demonstrated the presence of a phospho-monoesterase of type A_1 in cows' milk.

Folley and Kay (1937) define phosphatases as enzymes which catalyse the scission or formation of linkages (usually ester linkages) involving completely oxidised phosphoric acids. They are extremely widely distributed among both animals and plants, and the varying conditions under which they act have resulted in their being subdivided into various types. Phospho-monoesterase type A_1 shows an optimum activity at pH 9 to 10, and therefore in milk (pH 6·5 to 6·7) it is working considerably on the acid side of its optimum. It should be pointed out, however, that although phosphatase is invariably present in cows' milk (Folley and Kay, 1936), it has no obvious function in the milk itself and probably gains access to the milk from the mammary glands (Kay, 1925; Folley and Kay, 1935).

Phosphatase shows a regular variation in its concentration in milk through the lactation period, being at a minimum during the first 15 to 25 days and then steadily increasing. According to Davies (1939) milk contains from 7 to 20·8 mg per 100 ml of acid-soluble ester phosphorus, and Graham and Kay (1933) state that up to 50 per cent of these esters may be hydrolysed if milk is kept at body temperature for four hours. This action may, however, be readily suppressed by keeping the milk at low temperatures. Graham and Kay have also studied the distribution of phosphatase in milk, and they find that it is concentrated in the fatty layer, although in butter it is concentrated in the aqueous layer; they consider, therefore, that the phosphatase is associated with the protein layer surrounding the fat globules, and as soon as the nature of the emulsion changes, as in the manufacture of butter, this layer and the phosphatase are removed from the fat.

Chemical nature

Massart and Vandendriessche (1945) suggest that phosphatase is a heavy-metal protein (? Zn). It is inhibited by KCN and cysteine but not by fluoride.

Occurrence

Buruiana and Badlita (1940) in their book *Phosphatase in milk*, give values for milk of various species at different stages of lactation. They confirm the

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general findings of Kay and his collaborators. While the " alkaline " enzyme is most prevalent, human, goat and sow milk contain an " acid " phosphatase with a pH optimum of about 5. No phosphatase is apparently present in the milks of the ass and mare. These workers were unable to separate the enzyme from protein. According to Kannan and Basu (1948) phosphatase is high in the colostrum of cows, buffaloes and sheep, falls to a minimum value at 4 to 15 days and then rises steadily thereafter. They further report that Mn and Mg at appropriate concentrations can increase the phosphatase activity of cow milk phosphatase by 70 and 55 per cent (40 min. incubation), but buffalo, goat and sheep milk phosphatases are not activated by any inorganic ion. Anderson *et al.* (1949) in a comprehensive study have shown that milks vary in their phosphatase content and the rates of heat destruction vary in different milks. They consider that the destruction is not a reaction of the first order.

Hetrick and Tracy (1948b) conclude that most of the phosphatase in milk is located at the fat globule surface. They obtained the following values (as p.p.m. phenol:

whole milk	2,080
cream	10,000
butter	4,000
buttermilk	12,800
butter oil	nil
butter oil sludge	15,000
skim milk	1,280
whey	575

They suggest a formula—

$$\text{temperature } {}^\circ\text{F} = 174 - 9 \log \text{time (in secs.)}$$

for the inactivation of phosphatase, taking 1 p.p.m. phenol as an " end point ".

In a study of the acid phosphatases in cows' milk Mullen (1950) has found an enzyme hydrolysing phosphoric esters at pH 4·0. This phosphatase is remarkably stable towards heat, pasteurisation causing only about 15 per cent inactivation. It is, however, unstable towards light and in this way resembles the milk lipase which has recently been shown by Kay (1946) to be sensitive to sunlight and ultra-violet radiation. Chloroform, formaldehyde, and fluoride inhibit the enzyme, and the stimulating effect of magnesium is small.

Values for the content of cows' milk in this acid phosphatase gave titres varying between 2–8 Gutman acid phosphatase units per 100 ml. of milk. High values were found in early lactation, but there was no well-marked correlation between acid phosphatase and the concentrations of other milk constituents throughout the whole lactation, apart from the fairly close relationship between the enzyme and albumin + globulin nitrogen. In morning milk the acid phosphatase decreased as lactation advanced.

Mullen has also found that enzyme values are fairly constant for all four quarters in healthy non-mastitis-infected cows. Mastitis results in an increase in the acid phosphatase content in milk of the infected quarter, values as high as 50 units per 100 ml being obtained. As a suspension of *Str. agalactiae* is capable of hydrolysing phenyl phosphate at pH 4·1 it is possible that the mastitis-causing organism is largely responsible for the increased enzyme content of the infected milk.

For a survey of phosphatase tests for the efficiency of pasteurisation see Davis (1951a).

The chief application of phosphatase in dairy chemistry is in the control of milk pasteurisation, and this has come about as a result of the study of its thermal stability. The enzyme is stable for considerable periods under storage conditions, but is destroyed with increasing rapidity as the temperature rises. Kay and Graham (1933) found that 96 per cent is inactivated by holding at 63° to 65° C for 15 minutes, at 70° C for 3 minutes, and almost instantaneously above 75° C. Comparing these figures with those given by *Myco. tuberculosis*, the most heat-resistant of the pathogenic organisms likely to be found in milk, they also found that for a given time and a given temperature between 60° C and 75° C the organism was killed off completely before 96 per cent of the phosphatase had been inactivated. It therefore follows that a sample of pasteurised milk which gives no phosphatase reaction will contain no pathogenic organisms unless, through carelessness, they have gained access subsequently to the pasteurising process.

Reductases

" Reductases " were first observed in cows' milk by Duclaux. They are enzymes which bring about the reduction of certain organic substances. When the substances are coloured and change or lose colour sharply on reduction, it is possible to follow the process closely. Blyth (1901) used the decoloration of litmus in milk to indicate whether preservatives were present in milk or not and, therefore, indirectly to tell whether reductases were present, due to bacteria; fresh milk contains only a small quantity of reducing enzyme, but many micro-organisms secrete such an enzyme. At the present time, methylene blue is the substance considered most suitable to indicate bacterial content, as it is readily reduced and becomes colourless on reduction. This test is official for the Pasteurised milk, Tuberculin Tested milk and Accredited milk of the Milk (Special Designations) Order, 1949, of Great Britain. (See Statutory Instrument No. 1590, 1949, for raw milks, and Statutory Instrument No. 1589, 1949, for pasteurised milk. These are discussed in detail by Davis, 1951a.)

Schardinger's reaction. When methylene blue requires the addition of an aldehyde (formalin) before it is reduced by the reductase, the latter is called an " indirect reductase ", " aldehyde reductase ", or " Schardinger enzyme ". Schardinger (1902) found that fresh cows' milk will not reduce methylene blue alone, but will do so in the presence of formaldehyde. It is probable that Schardinger's reaction is due to an enzyme, as the reaction does not occur after the milk has been heated to 75° C for 20 minutes.

Hydrogenase, another reducing enzyme, produces sulphuretted hydrogen from sulphur. It is sometimes present in raw milk, but it is bacterial in origin and does not exist in fresh milk.

A reductase known as *xanthine oxidase* has been shown to be present in cows' milk by Morgan, Stewart and Hopkins (1922), and by Dixon and Thurlow (1924). This enzyme is capable of oxidising xanthine and hypoxanthine to uric acid at the expense of various oxidising agents (methylene blue, nitrates, etc.) and is identical with the Schardinger enzyme. Polonovski *et al.* (1947) have found that xanthine oxidase is only weakly active above 20° C but more active if the milk is cooled to 15° C or below. This change is irreversible and as milk fat solidifies at 15° C the authors suggest that the enzyme is released from the protein-fat linkage below this temperature. Surface active substances release the enzyme at higher temperatures. Worden (1943) has shown that xanthine oxidase activity is associated with the fat and increases on standing. Cooling and agitation also increase activity. He suggests that the protein carrier is part of the fat globule membrane and that the heightened activity is due to the rupture of the membrane. Keston (1944) finds that in the presence of xanthine and iodide, the Schardinger enzyme can liberate free iodine. The reaction is inhibited by thiourea. Kalckar and Klenow (1948) have isolated xanthopterin oxidase by fractional precipitation with ammonium sulphate. The enzyme was markedly inhibited by traces of pteroylglutamic acid.

The inherent reducing systems in milk

Saal and Heukelom (Eilers *et al.* 1947a) make a substantial contribution to the chemistry of the reducing systems in milk and conclude that oxygen, ascorbic acid and perhaps lactoflavin control the E_h[1] of fresh raw milk. Heating produces a reducing system of $E_0 + 0{\cdot}05$ v. Gould (1940b) finds that from 40–60 per cent of the total reducing power of milk is due to ascorbic acid (iodate method). Sulphydryl compounds could not be detected, and glutathione added to milk is rapidly destroyed (*ibid.*, 985). Heating to 80° C prevented this destruction, and added glutathione could then inhibit copper-catalysed oxidation (*ibid.*, 991). Crowe *et al.* (1948) consider that lactose is the source of all or part of the reducing system formed by the heating of milk. They describe a ferricyanide method for its estimation in which the pH is raised to 6·6 and the temperature lowered to 50° C. Ascorbic acid and sulphydryl groups are quantitatively not important.

Harland *et al.* (1949) have studied the reducing systems in milk powder and have shown that only part of the ferricyanide reducing systems reduce aneurin disulphide and that these are derived from the serum proteins. Although not destroyed as the fat is progressively oxidised, they appear to confer resistance to oxidation on milk powder. Aneurin disulphide and the nitroprussic reagents appear to measure the same reducing systems in heated milk.

An amperometric method has been used by Larson and Jenness (1950) for the titration of the sulphydryl groups in proteins using *o*-iodosobenzoate and iodine. They have obtained values of about 1·3 per cent (as cysteine) for lactoglobulin and nil value for casein. The same authors (*ibid.* 896) have studied the reducing systems of milk, using the same technique. They found a reducing system to be present in the fat phase, the serum proteins and the dialysable portion of the serum. They suggest that the fat globule membrane is responsible for the reducing system in the fat phase and that β-lactoglobulin is the principal reductant in the proteins and ascorbic acid is the chief reductant in the dialysate.

For further studies on this subject see Twigg (p. 209 in Wilson *et al.*, 1935).

Proteolytic enzymes

Many proteolytic enzymes have been described as growing in milk. The most important one seems to be tryptic in nature and is precipitated with the casein. The optimum pH appears to be 9·2.

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[1] The E_h value is the measure of the intensity of oxidising or reducing conditions in a system. Thus milk fat may be oxidised at an E_h of $+0{\cdot}4$ v, while a sour milk culture may have an E_h of $-0{\cdot}2$ v.

Lactase

This is an enzyme that converts lactose into glucose and galactose. Vandevelde (1908) demonstrated its presence in milk, although the amount is probably extremely small.

Aldolase

Polis and Shmukler (1950) have recently shown that aldolase, which splits fructose 1,6-diphosphate into dihydroxyacetone phosphate and phosphoglyceric aldehyde, exists in normal cows' milk in the same concentration range as in blood serum.

Amylase (diastase)

This is an enzyme which acts upon starch, breaking it down into dextrin. Other enzymes are known which convert dextrin into maltose (dextrinase) and maltose into dextrose (maltase). Amylase appears to be the most constant of all the enzymes of milk in its concentration. Colostrum and mastitis milk have a higher than normal content and human milk contains more than the milks of other mammals. Cream has a higher activity than skim milk and most of the amylase is precipitated with casein from milk. It has a maximum activity at pH 5·8 to 6·2 and is destroyed by heating to 60°–65° C for one hour. It has been proposed to use amylase activity as an index of the age of milk. Gould (1932) and Orla-Jensen (1932) have proposed the amylase reaction for the detection of heated milk.

Coagulase

Milk possesses the power to coagulate a solution of fibrinogen, and Buruiana (1940) has extracted the enzyme by ether from dried cream, followed by water extraction and acetone preparation. The pH optimum is 7. The coagulating power is strongest in ewe, bitch and human milk and absent from that of the sow.

(B) VITAMINS

The subject of vitamins in milk and milk products is too vast to be considered in this book. Reference may be made to Kon and Henry's article on "Nutritive aspects of milk" and Porter's article on "Vitamins" in Davis's *Dictionary of dairying* and also to the reviews on nutrition in the *Journal of Dairy Research*.

(C) OTHER MINOR CONSTITUENTS ("EXTRACTIVES")

Traces of nitrogen-containing organic substances have been reported to occur in milk, such as urea, uric acid and creatine.

Aceto (1947) finds 20 to 40 μg per cent as an average value for indican in human milk, and Spinelli (1947) reports 124 for cows' and 192 μg per cent for goats' milk. Karabinos and Dirrmer (1943) have reported the presence of hippuric acid in milk.

Neseni and Körprick (1948) have obtained values varying from 13 to 40 mg per cent for the residual N in milk. The values are usually higher at the beginning and end of lactation and are influenced by feeding.

Oxygen in milk and its effects

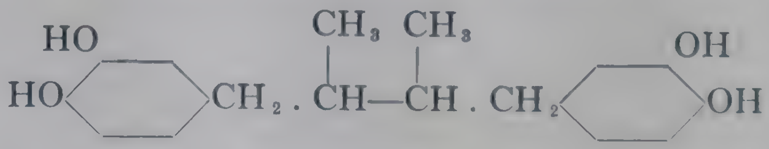
Milk from the udder contains about 6·6, 0·1 and 1·2 per cent by volume of CO_2, O_2 and N_2, respectively.

Guthrie (1946) has studied the oxygen content of milk and finds that though absent in udder milk, it increases up to 11 mg per litre in ordinary milk. De-aeration by 29 in. vacuum followed by ordinary bottling reduced the vitamin C from 17 to 13 in de-aerated milk as compared with 17 to 6 mg per litre in ordinary milk. The flavour was much improved, and oxidised flavour considerably reduced. Mixed raw milk has been found by Noll and Supplee (1941) to contain 0·47 (0·30–0·59) per cent O_2, 1·29 (1·18–1·63) per cent N_2 and 4·45 (3·44–6·28) per cent CO_2. Herreid and Francis (1949) give figures for dissolved oxygen in milk at different stages as follows: milking pails 4·5, churns 6·8, before pasteurisation 6·2, after pasteurisation 5·1, top of cooler 5·7, bottom of cooler 6·8, bottled 6·6 (mg litre approx. values).

Hartman and Garrett (1942) have successfully used the dropping mercury electrode for measuring the dissolved oxygen in milk. The milk solids did not affect the result. Sharp *et al.* (1941) have described a method for estimating dissolved oxygen in milk based on the oxidation of reduced ascorbic acid by oxygen in the presence of cucumber ascorbic acid oxidase.

In a study of the tocopherol and carotenoid contents of the milk of various breeds Krukovsky *et al.* (1950) found a relationship between the tocopherol content of the fat and the ability of the milk to resist the “ onset ’ of oxidised flavour. When the milk contained less than 2,500 μg of tocopherols per 100 g of fat it was likely to develop oxidised flavour during storage.

Morell *et al.* (1946) have described a method for determining the dissolved oxygen in butter fat. They report values of 2·6 (in air) and 12·3 (in oxygen) ml per 100 g fat. Chilson *et al.* (1949), in their studies of the role of ascorbic acid in the development of oxidised flavour in milk, found that addition of ascorbic acid could prevent the development of the flavour. Destruction of vitamin C by hydrogen peroxide also inhibited the reaction, but sunlight produced a marked flavour in milk to which ascorbic acid had been added. Stull *et al.* (1948a) have found that nordihydroguaiaretic acid (0·00125–0·0075 per cent)



will retard the destruction of ascorbic acid in ordinary milk. In the presence of 0·3 p.p.m. added copper it prevents the development of oxidised flavour for 5 days at 40° F (4·5° C).

Stull *et al.* (1951) have reported that nordihydroguaiaretic acid was most effective as an anti-oxidant at concentrations in the range from 0·001 to 0·01 per cent.

Citric acid, ascorbic acid and methionine were without effect when used alone, but in the presence of nordihydroguaiaretic acid they were active synergists. The anti-oxidant effect of the nordihydroguaiaretic acid was greatest at pH 6·5. It was considerably decreased at pH 3·0 and somewhat lessened at pH 8·0. Nordihydroguaiaretic acid alone, and also with citric acid will retard an oxidation which has already begun. The catalytic effect of the heavy metals proceeded in the following order: copper, nickel, iron, and cobalt, but the differences in activity were very slight.

Propyl gallate has been found to be effective as an anti-oxidant for refrigerated pasteurised milk by Chilson *et al.* (1950). Added at the rate of 20 mg per litre to freshly pasteurised milk the "onset" of oxidised flavour was prevented for 14 days at 35° F even in the presence of 0·5 p.p.m. of added copper. Ascorbic acid was not, however, stabilised by the gallate.

Stevens and Armour claim that cardboard or oxidised flavours in milk and milk products can be prevented by adding 1 part of pancreatin to 70,000 or 140,000 parts of milk or cream. The curd-forming properties of the milk are not affected (Brit. Pat. No. 546,593). Greenbank (1948) has published a review of work on oxidised flavours in milk.

For a more detailed dicussion of the chemistry of the constituents of milk the reader is referred to Davies's *The chemistry of milk* and the biennial reviews in the *J. Dairy Res.*, **19**, 226; **16**, 390; **13**, 93; **11**, 84; **9**, 95; **7**, 75 and **5**, 75.

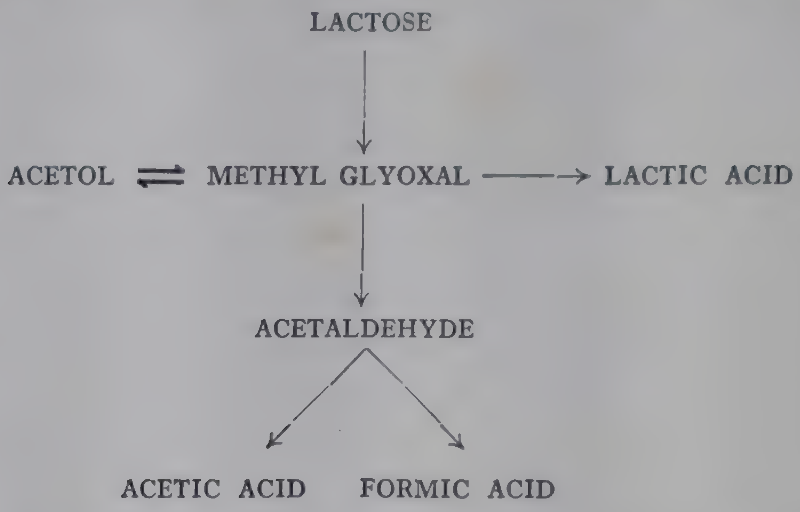
Effect of heat on the constituents of milk

Gould and Sommer (1939) find that milk heated momentarily to 76°–78° C has an apparent cooked flavour. The critical temperature was lowered by alkalinity and higher fat content and by sulphite. Copper sulphate retarded the appearance of the flavour. The flavour could be produced by addition of sulphite or glutathione. The source of the volatile sulphides was considered to be substances attached to the fat globules and passing into the buttermilk on churning. Andross (1939) has shown that the loss in boiling milk due to the residue in the pan and removal of skin amounts to 13 per cent on solids and nearly 16 per cent on calories. Patton and Josephson (1949) have isolated furfuryl alcohol from heated, but not unheated, skim milk. Patton (1950a) has isolated hydroxymethyl furfural from concentrated skim milk after prolonged heating. This appears to be derived from lactose, the presence of glycine, casein or its degradation products being essential, as the substance is not produced from pure lactose solution. This reaction is apparently associated with the browning reaction. Furfuryl alcohol and maltol were also formed.

The pH has a considerable effect in the production of furfural substances. Weakly alkaline solutions of lactose and condensed skim milk produce both furfuryl alcohol and hydroxymethyl-furfural. If acidified these materials produce hydroxymethyl-furfural but not furfuryl alcohol.

Bernhart and Linden (1950) have found that copper added to milk before heating to 100° C caused less oxidative effect (ascorbic acid destroyed) than if added afterwards. If the milk was heated only to 85° C the reverse held.

Heating milk for 1–2 hr. at 116° C increases the volatile acid content considerably. From 80 to 85 per cent of this is formic acid (Gould, 1945). Keeney *et al.* (1950) have also established the presence of acetol and acetic acid in heated milk (autoclaved condensed skim) and suggest the following scheme to account for the substances which have been reported to occur in heated milk—



Kass and Palmer (1940) have shown that the progressive browning of lactose solutions is accompanied by the production of acids, a small loss of " copper-reducing " power, a marked fall in optical activity and an appreciable conversion of lactose to ketoses. The general course of reactions was the same for all buffers. These workers conclude that the browning of milk can be satisfactorily accounted for by the absorption of the " lacto-caramel " by the colloidal caseinate, rather than by an amino group linkage between casein and lactose. Patton and Josephson (1949) have confirmed that prolonged heating at 90° C brings about a disappearance of the substances responsible for the nitroprusside reaction. The substances are stable in rennet whey and also in dialysed skim milk, but not in the latter if lactose is added. The disappearance is correlated with the caramelisation of the lactose. Miller and Sommer (1940) find a zone of maximum heat stability for skim milk at pH 6·4–6·5, stability falling off on both sides. The most sensitive region is pH 6·2–6·4, milk clotting at 90° and 155° C at these acidities. Cole and Tarassuk (1946) in a comprehensive study of heat coagulation found that milks from different cows varied widely, and the temperature–time values were not consistent between milks.

Haller *et al.* (1942) observed the following percentage reductions when goats' milk was pasteurised—

	160° F for 15 sec.	145° F for 30 min.
Soluble calcium ..	3·6	7·5
Soluble phosphorus ..	3·2	4·7
Soluble proteins ..	7·4	4·8
Curd tension (Hill) ..	5·1	38·4
,, ,, (D.S.A.)	6·9	48·4
Ascorbic acid	0	39·5

Strogill *et al.* (1951) employing the method of Choi *et al.* (1946) have shown that if milk is heated at 96° C ammonia is produced at an average rate of 0·96 mg per cent per hour in an open flask, and 1·25 mg per cent per hour when the milk is heated in sealed containers. At the point when milk turns brown and develops a caramel flavour the increase in ammonia may amount to 1·5 to

2·0 mg per cent. Although ammonia production appears to run parallel to the production of cooked flavour it must not be assumed that the two can be directly correlated. Most of the ammonia appears to be derived from the known casein nitrogen.

Stewart (1951) has used porphyrexide, a powerful oxidising agent, to estimate the heat-produced reducing compounds in milk products, using *p*-aminodimethylaniline sulphate as an indicator. The results correlated well with the intensity of cooked flavour. The efficiency of this method can be seen from the fact that the E_h of porphyrexide at pH 7·0 is + 0·725 v compared with the value for 2,6-dichlorophenolindophenol of + 0·217 v.

Greenbank and Wright (1951) have shown that if milk is de-aerated before heat treatment there is a greater fall in O/R potential and also an increased amount of reducing compounds formed in the milk when it is heated. De-aeration of milk before heat treatment was found to result in a milk powder which developed tallowy flavour more slowly than usual.

Hájek (1950) has reported the following time–temperature combinations for the inactivation of enzymes in milk—

Aldehydreductase: A few minutes at 70° C and instantaneously at over 80° C.
Amylase: 1 hr. at 57° C, 30 min. at 58° C, 14 min. at 59° C and 6 min. at 60° C.
Lipase: 50 to 115 min. at 62° C and instantaneously at over 67° C.
Monobutyrase: Instantaneously at 67° C.
Peroxidase: 2 hr. at 70° C, 20 min. at 72° C and 7 min. at 74° C.
Phosphatase: 55 to 65 min. at 60° C, 20 to 30 min. at 62° to 63° C, and in a few seconds at over 67° C.

Pien (1945) has given similar data for peroxidase and the Schardinger enzyme.

General

For a general account of recent advances in dairy chemistry see the reviews of dairy chemistry in the *Journal of Dairy Research* (Aschaffenburg and Rowland 1952, Aschaffenburg & Rook 1949, Scott Blair 1949, Rowland & Scott Blair 1942, Rowland & Scott Blair 1940, Davies 1938, Davies 1936a, and Davies 1934).

CHAPTER 3

VARIATIONS IN THE COMPOSITION OF MILK

In a previous chapter various results have been given showing the average composition of milk. It was there pointed out that the extremes show somewhat wide variations from this average. These variations may be due to many causes. Among the factors which have, from time to time, been alleged to have some influence on the composition of milk, particularly on the fat content, the following may be included—

(*a*) the number of animals in the herd
(*b*) the breed
(*c*) the individuality of the cows
(*d*) the health of the animals
(*e*) the type of feeding
(*f*) the age of the animals
(*g*) climate
(*h*) season
(*k*) the stage of lactation
(*l*) the frequency of milking
(*m*) the intervals between successive milkings
(*n*) the personnel of the milkers
(*o*) sexual excitement
(*p*) variations in yield

It is well known that differences in the milking periods alter the fat content of the milk considerably. Collins has computed that a change of one hour in the milking period will alter the percentage of fat in the milk by 0·2; when the interval is longer the amount of fat is less, and vice versa. This computation is, of course, only approximate and moreover, to be of any value it implies that where the intervals are of the same length, the percentage of fat in the milk will remain unchanged. Unless the amount of fat present in the milk of a given herd is, in fact, fairly constant, no such computation would be possible.

Many experiments have been carried out with different herds of cattle to see what effect, if any, a change in the condition of the cattle would have upon the composition of the milk produced. These tests are usually carried out by dividing the herd into two portions and using one portion for the experiment and the other for the control. Any differences which are found between the milk of the one portion and that of the other are attributed to the effect of the particular condition which has been changed. All this tacitly assumes that there would not have been any change in the composition of the milk had the conditions not been altered. There is, therefore, a considerable body of opinion which believes, perhaps subconsciously, that the milk of a given herd kept under normal conditions will change very little in composition from day to day, or that where such a change does take place, it is gradual and not sudden.

It is proposed to deal with the variations, if any, which have been thought to be due to each of these factors *seriatim*.

(a) The number of animals in the herd

If a composite sample of the whole of the milk from all the cows in the world could be obtained, it would probably have a composition very nearly equal to: fat 3·75 per cent, solids-not-fat 8·9 per cent. The milk of individual cows, however, may give results very different from these figures. It will readily be seen, therefore, that the composition of the milk from a herd of cows will, in general, be nearer the average the larger the number of animals and the more varied the type. Tocher (1925) has calculated that the milk of a random herd of ten cows is likely to contain less than 11·5 per cent of total solids in only one case in two thousand.

Any other factors which have been found to have an effect on the composition of the milk, e.g. breed, period of lactation, or age, will still produce their usual effect unless, as does not often happen, these factors are more or less evenly distributed throughout the herd. In dealing, therefore, with the likely effects caused by the number of animals, the other factors must be taken into account.

(b) The breed

The breed of the cow is a very important, probably the most important, factor controlling the composition of milk. Wide differences between different breeds have been observed, and substantiated by other workers. It is impossible, however, to give exact figures for each breed, as published figures are neither particularly numerous nor perfectly consistent, although certain general differences have been noted. On the average, Friesians give the lowest percentage of both fat and solids-not-fat, and Channel Island stock the highest. Some published results are given below—

Table 3.1—Milk from different breeds (*American observers*)

Breed	Observer			
	Collier		Lythgoe (1914)	
	Fat per cent	Solids-not-fat per cent	Fat per cent	Solids-not-fat per cent
Jersey	5·61	9·80	5·65	9·10
Guernsey	5·12	9·47	5·23	9·37
Devon	4·15	9·59	—	—
Holderness	3·55	9·10	—	—
Ayrshire	3·57	9·45	4·01	8·63
Holstein	3·46	8·96	3·41	8·28

Richmond gives the following figures—

Table 3.2—Fat percentage in milk of cows of different breeds

Percentage of fat	Dairy Shorthorn	Pedigree Shorthorn	Kerry	Jersey	Red Polled	Other breeds	TOTAL
Above 10	1	—	2	—	—	—	3
8 to 10	10	—	6	11	—	1	28
7 ,, 8	11	3	17	36	—	4	71
6 ,, 7	76	8	111	113	6	21	335
5 ,, 6	382	91	408	136	41	45	1,103
4 ,, 5	1,313	594	659	89	70	70	2,795
3·5 to 4	625	362	182	14	31	43	1,257
3·0 ,, 3·5	309	173	84	3	26	34	629
2·9	28	15	6	—	6	4	59
2·8	25	15	7	—	1	5	53
2·7	21	8	7	—	2	3	41
2·6	16	10	2	—	2	1	31
2·5	5	5	—	—	1	—	11
2·4	8	5	1	1	—	1	16
2·3	5	1	—	—	—	2	8
2·2	2	—	—	—	—	1	3
2·1	5	1	—	—	—	1	7
2·0	2	—	—	1	—	—	3
1·9	2	1	—	—	—	—	3
1·8	—	—	1	—	—	—	1
1·7	—	—	—	—	—	1	1
1·6	1	—	—	—	—	—	1
1·5	—	—	—	—	—	—	—
1·4	—	—	—	—	—	1	1
1·3	1	—	—	—	—	—	1
1·2	—	—	—	—	—	—	—
1·0	1	—	—	—	—	—	1

The composition of the samples showing the highest and lowest fat percentage was —

Total solids	20·97	11·55
Fat	12·52	1·04
Ash	0·73	0·85
Solids-not-fat	8·45	10·51
Authority	Bannister	Richmond

Table 3.3—Solids-not-fat percentage in milk of cows of different breeds

Percentage of solids-not-fat	Dairy Shorthorn	Pedigree Shorthorn	Kerry	Jersey	Red Polled	Other breeds	Total
Above 10	21	—	6	15	2	7	51
9·5 to 10	112	37	88	91	16	47	391
9·0 ,, 9·5	972	390	744	200	69	114	2,489
8·5 ,, 9·0	1,491	734	594	91	76	59	3,045
8·4	108	70	23	2	6	6	215
8·3	62	30	22	2	7	2	125
8·2	36	9	6	2	—	1	54
8·1	12	9	3	1	—	1	26
8·0	15	7	—	—	3	—	25
7·9	10	2	2	—	1	1	16
7·8	5	1	—	—	—	—	6
7·7	3	2	—	—	2	—	7
7·6	2	1	1	—	—	—	4
7·5	—	—	—	—	2	—	2
7·3	—	—	—	—	1	—	1
7·1	—	—	—	—	1	—	1
6·6	—	—	1	—	—	—	1
6·2	—	—	1	—	—	—	1
6·1	—	—	1	—	—	—	1
4·9	—	—	1	—	—	—	1

Table 3.4—Percentage solids in milk of cows of different breeds (*Vieth*)

Breed	Total solids			Fat			Solids-not-fat		
	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Dairy Shorthorn	18·7	10·2	12·90	10·2	1·3	4·03	10·6	7·6	8·87
Pedigree ,,	16·8	10·5	12·86	7·5	1·9	4·03	9·8	7·6	8·83
Jersey	19·9	11·0	14·89	9·8	2·0	5·66	10·4	8·1	9·23
Kerry	18·6	10·6	13·70	10·5	1·8	4·72	10·6	4·9	8·98
Red Polled ..	16·2	9·7	13·22	6·6	2·5	4·34	10·2	7·1	8·88
Sussex	17·4	11·5	14·18	7·6	2·9	4·87	10·3	8·4	9·31
Montgomery ..	16·1	10·2	12·61	6·5	1·4	3·59	10·0	7·9	9·02
Welsh	17·6	11·9	14·15	8·3	3·0	4·91	9·6	8·9	9·24

The Essex County Milk Recording Society published the following figures for their 1920–1922 averages—

Table 3.5—Fat percentage, different breeds

Breed	Morning milk	Evening milk	Mixed milk
Friesian	3·03	3·83	3·36
Crossbred	3·16	3·95	3·48
Shorthorn	3·42	4·08	3·70
Red Poll	3·64	4·36	3·92
Channel Island	4·34	5·85	4·95

Overman *et al.* (1929) give the following averages—

Table 3.6—Composition of milk from different breeds

	Average per cent of	
Breed	*Fat*	*Solids-not-fat*
Holstein	3·55	8·97
Ayrshire	4·14	8·94
Guernsey-Holstein	4·37	9·39
Jersey	5·18	9·51
Guernsey	5·19	9·68

Drakeley (1927) has worked out averages from 6,566 analyses of milk obtained at forty of the B.D.F.A. Dairy Shows—

Table 3.7—Composition of milk, different breeds (*Drakeley*)

	Average per cent of	
Breed	*Fat*	*Solids-not-fat*
Jersey	5·18	9·30
Guernsey	4·88	9·29
Kerry	4·30	9·25
Dexter	4·15	9·11
South Devon	4·02	9·09
Ayshire	3·97	9·09
Red Poll	3·81	9·04
Dairy Shorthorn	3·78	9·00
Lincoln Red	3·76	9·00
British Friesian	3·67	8·78

Tocher (1927) has arranged his 676 random samples in averages for the fat in the milk of each breed in the following way—

Table 3.8—Fat percentage, different breeds (*Tocher*)

Herd	*Average per cent of fat*
Jersey	5·43
Guernsey	5·16
Kerry	4·67
Welsh	4·40
Ayrshire	4·09
Shorthorn	3·91
British Friesian	3·63

Table 3.9—Average values from pedigree herds (*Bartlett*, 1950)

Breed	Annual yield (lb.)	Fat per cent	S.N.F. per cent
Ayrshire	7,215	3·6	9·05
British Friesian	8,363	3·47	8·74
Dairy Shorthorn	6,597	3·63	9·08
Devon	5,340	3·88	9·13
Dexter	4,115	3·83	9·06
Guernsey	6,882	4·53	9·26
Jersey	6,611	4·86	9·20
Kerry	6,652	3·90	9·12
Lincoln Red Shorthorn ..	6,539	3·52	9·02
Red Poll	7,113	3·68	9·03
South Devon	6,011	3·81	9·16
Welsh Black	5,128	—	—

Davis (1952a) gives a survey of reported breed averages.

(c) The individuality of the cows

Apart from the large variations which occur in the average composition of the milk of cows of different breeds, considerable variations occur in the milk of different animals of the same breed. Thus, in the reference quoted above (Overman *et al.* 1929) are given the following extremes—

Table 3.10—Variations in the milk of cows of the same breed

Breed	Fat per cent	Solids-not-fat per cent	Total solids per cent
Ayrshire	2·92 to 5·66	7·20 to 10·34	10·56 to 15·76
Guernsey	3·65 ,, 7·66	8·19 ,, 11·10	12·07 ,, 17·88
Holstein	2·60 ,, 6·00	7·82 ,, 11·90	10·72 ,, 17·62
Jersey	3·28 ,, 9·37	7·68 ,, 11·07	10·96 ,, 17·68
Guernsey-Holstein ..	2·72 ,, 7·50	7·86 ,, 11·73	10·58 ,, 17·90

Tocher (*loc. cit.*) found the figures for 341 samples of the milk of individual Ayrshire cows to vary from 7·00 to 10·66 per cent of solids-not-fat and from 2·30 to 7·50 per cent of fat.

(d) The health of the cows

The available evidence seems to show that the ill-health of the animal has far more effect on the yield of milk than upon its composition. The yield is often diminished, sometimes to a very considerable extent. The most important disease in this respect is mastitis or inflammation of the udder, which is discussed in Davis's *Dictionary of dairying*. The effect on the fat percentage is variable (Minett and Martin 1936) but the solids-not-fat is almost invariably reduced, chiefly due to the fall in lactose and its replacement by an isotonic equivalent of sodium chloride (Foot and Shattock 1938, Rowland and Zein-el-Dine 1938 and 1939, Rowland 1951a).

Rowland (1951a) is of the opinion that mastitis is not a major factor in the composition of the country's milk supply as a whole, but Davis has observed that notorious cases of herd bulks being low in s.n.f. are often associated with extensive mastitis in a Friesian or Shorthorn–Friesian herd. Surveys about 1939 indicated that about 35 per cent of English dairy cattle were suffering from mastitis (mostly sub-clinical) but the widespread use of penicillin since 1945 has resulted in a decline in this figure to about 13 per cent. Investigations on herds whose owners have asked for assistance in combating mastitis will give an incidence of about 20 per cent, but this figure is, of course, not true for all dairy cattle (Stableforth 1950).

(e) The type of feeding

The first impression obtained on reading through the relevant literature is that it is almost universally agreed that where a herd is already receiving a well-balanced ration, a change in the feeding will not cause any material change in the quantity or quality of the milk. A ration appreciably heavier than that appropriate to the milk yield may slightly increase this yield, but has no effect on the percentage of fat in the milk; it is far more likely to lead to an increase in the live weight of the animal. If, on the other hand, a herd is not receiving an adequate ration, an improvement in the kind and quality of the feed will lead to an increased output.

The results of an interesting experiment carried out at the Cornell University Experimental Farm in 1921 are suggestive. Milk and milk-fat records were kept for a complete year of a herd of seven poorly-nourished cows owned by a local farmer. The herd was then removed to the Cornell University Experimental Farm and liberally fed for two years. For the fourth year it was returned to the farmer and fed and managed as during the first year. The following records were obtained—

	1st and 4th years	*2nd and 3rd years*
Average weekly yield of milk per cow	109 lb	155 lb
Average weekly yield of fat per cow	4·7 lb	7·1 lb
Average percentage of fat in milk . .	4·31	4·58

Thus, although there was nearly a fifty-per-cent improvement in the milk yield due to the improved conditions, the increase in the percentage of fat in the milk was only a little more than 6 per cent (a numerical difference of 0·27).

Effect of grazing

Some workers, however, have held that there is one change in feeding which does cause a material alteration in the quantity and quality of the milk produced, and that is the turning of the cows out to grass in the spring.

R. O. Davies and Provan (1927) examined the milk of six individual cows of a herd both before, and a fortnight after, the commencement of grazing. The following results were obtained, the figures being rounded off to one place of decimals—

Table 3.11—Percentage composition of milk before and after grazing

Cow	Protein		Fat		Solids-not-fat	
	Before grazing	After grazing	Before grazing	After grazing	Before grazing	After grazing
1	3·1	3·4	3·8	3·8	9·6	9·3
2	3·3	3·4	3·6	3·8	9·1	9·2
3	3·5	3·6	3·8	3·7	9·1	9·0
4	3·3	3·7	3·4	4·0	9·0	9·1
5	3·8	4·0	3·9	3·2	9·6	9·9
6	3·3	3·4	3·0	3·4	8·6	8·7
Average	3·4	3·6	3·6	3·7	9·2	9·2

These results indicate that on the average there has been a slight rise both in protein and in fat; the solids-not-fat remain unaltered. These figures, however, do not necessarily give a true picture of the maximum change which takes place immediately the cows are put out to grass, since the milk was not sampled until a fortnight afterwards, and some recovery may have taken place during this time.

R. O. Davies and Provan (1928) carried out an experiment with a herd of 14 cows, the cows being treated individually. Here again, sampling was not continuous over the period when the cows were put out to grass, being carried out immediately before and a fortnight after grazing commenced. The following are the mean fat contents of the morning and evening milks before and after grazing (figures correct to one place of decimals)—

Table 3.12—Percentage fat in milk before and after grazing

Diet before grazing	Morning milk		Evening milk	
	Before grazing	After grazing	Before grazing	After grazing
Normal	3·3	3·0	3·5	3·2
Low protein	3·5	3·5	3·4	3·3

Under these circumstances a fall in fat not exceeding 0·3 per cent was observed. Again, however, this does not necessarily show the full effect of the *change* of diet, as some of the effects may well be less marked after a fortnight. On the other hand, the change, if any, may be progressive and may amount to very little indeed in the first few days.

R. O. Davies and Pugh (1929) continued this work and came to certain conclusions, but they considered that the number of cows which had been under observation was not sufficient to enable detailed interpretation of the results to be made. They consider, however, that it is clear that there is a connection between the change in the total amount of fat and that of the phosphorus-containing compounds at the commencement of summer grazing, and that the effect produced by grazing depends upon the winter feed. Where the cows are on a high level of nutrition immediately before being put out to grass and are turned on to grass of poor quality, there is a diminution of fat secretion. When, on the other hand, the animals are on a low level of nutrition before grazing and are given sufficient pasture of good quality, an increase in fat secretion takes place.

Golding *et al.* (1932 and 1935) carried out, during five years, an investigation of the milk of a typical herd of about ten Shorthorn cows kept under South of England conditions. The fat percentage was determined each day, but the detailed figures published are weekly averages. For five consecutive years the duration of the test included the times when the cows were put out to grass. In the following table the results for the whole five years have been grouped to show the immediate effect of grazing (i.e. the average sample taken next after grazing commenced, compared with the sample immediately before) and also the maximum change between the sample taken immediately before grazing and the next four samples after grazing—

Table 3.13—Alteration in percentage of fat due to grazing

	Time	Year				
		1929	1930	1931	1932	1933
Immediate effect	a.m.	0	0	−0·1	−0·1	−0·3
	p.m.	0	−0·1	−0·1	+0·2	−0·1
Maximum effect during one month	a.m.	−0·2	−0·1	−0·3	−0·5	−0·5
	p.m.	+0·2	+0·1	−0·2	+0·2	−0·3
Weeks after grazing commences in which maximum change observed	a.m.	4	3	3	4	3
	p.m.	2	1	3	1	3

From these results it appears that there is some evidence for a diminution of the percentage of fat in milk during the first week that the cows are out at grass. The amount of variation is, however, slight and scarcely more than may normally occur in a herd in which there are no alterations of feed. The maximum change during one month is still, on the whole, not large, although rather more than that noticed during the first week. This maximum change is by no means identical from year to year, and moreover it does not occur in any particular week after the change in the food, although there is a tendency for the greatest decrease to occur during the third week after grazing commences. On the whole, the investigations of Golding *et al.* suggest that the change to grazing is accompanied by a much less violent change in the composition of the milk than was formerly supposed.

The one author whose results appear to be outstanding is Sommerfeld (1935) who states that the fat content dropped from 3·2 per cent to 2·2 per cent when the cows were turned out to grass. Sommerfeld does not regard this change as unavoidable, as he indicates methods of prevention.

General effect of diet

The general effect of feeding has been well summed up in Bulletin No. 16 of the Ministry of Agriculture and Fisheries. The following extracts are taken from this source: " It is commonly maintained by practical men that the foods fed to cows exercise a considerable influence on the percentage of fat in milk. On the other hand, research workers who have devoted a great deal of attention to this question have been unable to find any evidence to support this view. . . . "

" The results of numerous experiments, carried out under varying conditions in all parts of the country, show that such changes as can be effected in the composition of the milk of suitably fed cows are slight and temporary. . . . It remains broadly true to say that where cows are suitably fed, milk cannot be altered appreciably in respect of the percentage of butter fat by the foods, and that as regards the solids-not-fat the effects are still more difficult to trace." A little later in the same bulletin, after an experiment has been described, we read: " It is evident from this experiment that while starvation considerably affects the quantity, it only to a slight extent affects the quality in respect of the percentage of fat. . . ."

Lauder and Fagan (1914) state that within wide limits the composition of milk is very little affected by the nature of the food supplied, whilst Cranfield (1927) states that normal feeding appears to have but little influence on fat percentage, although some workers claim positive results in this direction. A badly balanced ration, or insufficient food bordering on starvation, will cause a decided change in the normal percentage of fat, but in such cases the health of the animal usually suffers.

Effect of particular types of foods

Some of the available evidence dealing with the effects which may follow the ingestion of particular types of food will now be considered. For convenience the subject will be divided into several sub-headings: (1) sugar beet residues; (2) minerals and salts; (3) coconut and palm-kernel cake; (4) oils and fats; (5) proteins; (6) watery foods.

(1) *Sugar beet residues.* Cranfield (1929) found that sugar beet pulp produced a higher yield of milk but that the average composition was unaltered. Zwagermann (1933) found that feeding with sugar beet tops and leaves did not alter the fat content of the milk, although it produced an unpleasant odour and taste. Roemmele (1934) by substituting potato distillation residue for beets, caused an increase in milk yield and in fat yield.

(2) *Minerals and salts.* Liebscher (1930) found that a supplementary mineral ration did not affect the composition of the milk. Hansen (1930)

states that the administration of iodised salt was not followed by any increase in the yield of milk or in the live weight of the animal, and Scharrer (1927) found that the daily administration of either 7·5 or 15 mg of iodine was without striking effect on the milk yield or on its fat content. Zaykowsky (1926) found that the addition of either chalk or calcium phosphate in the diet raises the calcium and phosphorus content of the milk, and at the same time increases the fat content and the yield; but, on the other hand, Mattick and Wright (1925) found that calcium chloride, ammonium chloride, sodium bicarbonate and sodium phosphate, when administrered to cows, do not alter the calcium content of the milk, nor do they alter the composition; whilst Lauder and Fagan (1914) in a particular experiment, showed that the ingestion of calcium phosphate had no apparent effect upon the composition of the milk.

(3) *Coconut and palm-kernel cake.* Honcamp *et al.* (1930 and 1931) have shown that feeding with coconut or palm-kernel and coconut cake increases the content of fat, which is particularly marked where the amount of oil present is high. Considerable variations in the effect on individual cows were, however, observed. The favourable effects appeared to be confined to those cakes which contained more oil than is usual, certainly more than 5 per cent. Kronacher *et al.* (1933) also found that the use of palm-kernel cake increased the fat content by from 0·2 to 0·7 per cent but did not increase the yield.

Crowther and Woodhouse suggest that the typical acids of palm-kernel oil had increased in the milk-fat when a portion of the feed consisted of cake containing this oil.

(4) *Oils and fats.* It appears that, apart from differing effects on different animals, the kind of oil used exerts a considerable influence. Thus, Sutton *et al.* (1932) found that 1 lb. of maize oil per day did not affect the milk yield or the percentage of fat, but it changed the composition of the fat. Similar results were obtained by Sheehy (1933 and 1934) in the case of cotton-seed, olive and soya oils.

Moczarski and Bormann (1935) by means of experiments carried out by feeding very rich, medium and fat-free rape cake, were led to the conclusion that the excess of fat in the cake did not diminish the milk yield when fed below 0·1 per cent of the cow weight, but it appeared to diminish the production of fat. When fed above 0·1 per cent of the live weight it diminished both the yield and the quality of the milk. Sheehy (1923) considered that if the diet were deficient in fat, the maximum milk fat production could not be obtained, no matter how liberal the diet. Maynard *et al.* (1934) found that additions of more than 4 per cent of fat to a grain mixture ration did not significantly increase the fat or the total-solids, whilst the feeding of milk or cream to cows was found by Allen to increase both the total production of fat and the percentage of fat in the milk produced. Maynard and McCay (1933) found that when the cows were fed on a ration in which most of the oil was replaced by starch, both the milk and the fat yields were heavily decreased.

The evidence for the effect on the composition of the milk of feeding fat to cows is, like the evidence deduced from many types of feeding experiments, somewhat contradictory, but the evidence that some change is produced by means of cod liver oil is far more unanimous. Peterson (1932) found that eight ounces of cod liver oil per day reduced the percentage of fat in the milk without altering the yield. Sheehy (1933) found that whilst cotton-seed, olive and soya oils up to 30 oz. daily had no effect on the composition of the milk, from 6 to 8 oz. of cod liver oil (or 15 oz. of linseed oil) had a depressant effect.

Golding *et al.* (1928) have reported that 6–8 oz. cod liver oil daily depressed the fat content of the milk and this effect persisted for six weeks.

Brown and Sutton (1931) found that oil appeared to reduce both the yield and the quality of the milk. Indeed there is a fairly widespread opinion that excess of oil in a ration does tend to lower the amount of fat produced. The extent of the change is, however, not great unless very large (and unwise) quantities of oil are used.

(5) *Proteins.* There is little doubt that a serious deficiency in proteins in the feed will cause a depression in the yield, in the fat content, or in both of these. Walker (1923) has given an account of an improvement which took place in the composition of a very poor milk by increasing the amount of protein (which had been too low) in the feed. Perkins (1933) found a slight reduction in the fat content by low protein feeding, but Harrison and Savage (1933) stated that they found no evidence that increased protein, beyond that really necessary, stimulated milk production, and it is agreed that it is only where the protein intake becomes really low that any serious alteration in the milk-supply takes place. Harrison *et al.* (1934) showed that no significant improvement in milk yield was produced by raising the protein level of the ration to more than sixteen per cent.

Since the last edition of this book was published, well-controlled experiments at Shinfield and also in New Zealand have proved conclusively that under-feeding reduces the solids-not-fat. Thus in one experiment the normal s.n.f. was 8·68, on a low protein ration 8·50, and on a low energy ration 8·34 per cent (Rowland 1951a). The fat content was not affected.

A recent finding of great interest is that a low roughage or fibre content of the ration seriously reduces the fat content (Balch *et al.* 1952). A claim that low magnesium can be a cause of low fat values has not been confirmed by carefully controlled experiments.

Dunlop (1951) has obtained a positive response in butter-fat production when 10 g copper sulphate was added to the evening concentrate from 1 to 10 days before the time of the analysis.

It is stated that the low fat percentage of the milk of certain animals exhibited at the London Dairy Show has been shown to be due to a deficiency of copper.

(6) *Watery foods.* If there is one section of this subject on which " common knowledge " used to be almost unanimous, it was on the supposed fact that watery foods, or the drinking of more than the usual quantity of water, cause the secretion of a larger bulk of poorer milk; yet no opinion receives less support from the evidence available. All published investigations agree that feeding watery foods fails to lower the quality of the milk.

Investigations on the effect of diet are of course difficult to carry out on the usual basis of a scientific experiment, i.e. to have only one variable at once, because the living organism is very complex, and we have little knowledge of the nature of the variables. It need not be deduced from this that such experiments are worthless, but it should lead to caution in drawing definite conclusions from isolated reasearches. When a considerable number of investigations all lead to more or less the same results, there would seem to be good ground for their acceptance; and it must be agreed that the available evidence does not support in any way the widespread opinion that the composition of milk is greatly influenced by various changes in the environment of the animals, and in particular by alteration in the diet.

(f) The age of the animals

Other things being equal, the older the animal the less the percentage of fat in the milk. This was well shown by Crowther (1905) on the results obtained during six months with 903 Ayrshire cows of approximately equal periods of lactation—

Table 3.14—Fat percentage: different ages of cows

Age of cows—years	*No. of cows*	*Average fat per cent*
2	30	3·83
3	147	3·87
4	164	3·76
5	137	3·66
6	110	3·63
7	88	3·63
8	80	3·69
9	50	3·63
10	36	3·64
11	28	3·60
12	16	3·48
13	10	3·42

The same type of result was found by Tocher in his 676 random samples, and similar conclusions have been reached by Eckles and Palmer (1917); cf. also Kay and MacCandlish (1929).

Bartlett (1934) has worked on the composition of the milk of 300 complete lactations of the individual cows of a herd, each sampled once a month. Some 10,000 samples were examined, of which 80 per cent were obtained from Shorthorns and 20 per cent from Guernseys. As a result of this work Bartlett has confirmed many previous findings.

Immediately after calving, the milk is rich in solids-not-fat, but the concentration falls rapidly and a fairly constant level is reached about 15 days after calving. This level is maintained for most of the lactation period, but a continuous rise becomes evident over the last three months of lactation.

When cows are milked once daily, at the end of the lactation period, a rapid decrease in the solids-not-fat content of the milk tends to occur.

The solids-not-fat are lower in the second lactation than in the first by some 0·2 per cent, whilst subsequent lactations tend to give still lower results.

In a later paper, Bartlett (1934) states that as the number of lactations increases from one to nine, the total yield of milk throughout each period increases until the seventh lactation, after which it falls; the yield during the ninth period is little more than that during the first.

Both the fat and the solids-not-fat decrease in percentage as the number of lactations increases, although there is a tendency for the percentage of fat to increase slightly after the seventh pregnancy.

The effects of age, stage of lactation, season and other factors have been studied by Bailey, G. L. (1952).

(g) The climate

It is not easy to separate the influence of climate from all the effects which tend to fix the amount of fat in milk, particularly that of breed. To decide the question definitely it would be necessary to take the averages of the fat content of the milk of different herds of the same breed of cattle situated in different countries. There is very little evidence of this type available, but Tocher's figures on Scottish cattle and Overman, Sanmann and Wright's figures for American cattle of the same breed are not without interest—

Table 3.15—Fat percentage: influence of climate

	Average percentage of fat	
Breed	*O. S. & W.*	*Tocher*
Jersey	5·18	5·43
Guernsey	5·19	5·16
Ayrshire	4·14	4·09
Friesian	3·55	3·63

It would be necessary to produce a much greater number of figures than these to decide the question finally, but what evidence there is does not point to any very serious effect of climate on milk composition. The average amount of fat in cows' milk in a hot country, such as India, is not very different from that in a cold country like Canada.

Generally speaking, the effects of weather conditions on yield and quality are comparatively slight and temporary. A change to a decidedly high or a decidedly low temperature tends to reduce the yield of milk, the fat percentage remaining the same or being even slightly increased.

(h) The season

It is often stated that season has a considerable effect on the fat content of milk. By many this has been attributed to the fact that, in this country, cows are usually put out to grass about the beginning of May, and that the young grass has the effect of lowering the percentage of fat in the milk. The average figures for the percentage of fat in milk for each of the 12 months of the year are given in Table 3.16, which has been compiled on the basis of information obtained from the Public Analysts of the places named.

It appears from this table that sending cows out to grass has the temporary effect of lowering the quantity of fat in the milk. It is, however, by no means sure that such is actually the case. As will be seen from the next section, the stage of lactation has a pronounced effect on the percentage of fat, and it is more than likely that the smaller amount of fat normally present in milk during the months of April, May and June is due far more to the fact that a very large proportion of the milch-cow population is in the earlier stage of lactation during these months. This suggestion is further borne out by the fact that the change is progressive. From the highest month, which is November, the fat gradually falls month by month until June, when it is at its lowest, after which there is

Table 3.16—Percentage of fat in milk: monthly averages for several years

Month	Birmingham City	Kent County	Portsmouth City	Durham County	Salford City	Kingston-upon-Hull	Somerset & Dorset com'cial samples
January ..	3·66	3·87	3·64	3·59	3·60	3·68	3·78
February ..	3·57	3·84	3·54	3·53	3·54	3·64	3·61
March ..	3·54	3·72	3·55	3·63	3·56	3·65	3·51
April ..	3·52	3·77	3·53	3·53	3·57	3·67	3·49
May ..	3·46	3·70	3·46	3·58	3·56	3·55	3·50
June ..	3·40	3·63	3·47	3·38	3·46	3·41	3·52
July ..	3·51	3·76	3·63	3·44	3·53	3·56	3·61
August ..	3·61	3·79	3·66	3·47	3·64	3·63	3·72
September	3·71	3·79	3·75	3·51	3·74	3·73	3·82
October ..	3·84	3·96	3·72	3·76	3·87	3·87	4·01
November..	3·87	3·99	3·82	3·82	3·86	3·81	4·16
December	3·78	3·94	3·78	3·74	3·59	3·72	4·00
Total no. of samples	44,730	21,264	7,596	4,078	13,960	10,253	88,608

a steady rise again until November. A very much more rapid change than this would be expected were the whole of it due to putting out the cows to grass. In all probability the season of the year has little effect on the composition of milk.

In sub-section (*e*) of this chapter the positive evidence for the assumed change of fat percentage due to early spring grass has been collected. It is

Table 3.17—Monthly average composition of milk: all areas, 1910–1934

Month	No. of samples	Fat per cent	Solids-not-fat per cent	Total solids per cent
January	55,226	3·65	8·79	12·44
February	55,631	3·58	8·77	12·35
March	60,470	3·54	8·76	12·30
April	57,235	3·52	8·73	12·25
May	65,357	3·46	8·79	12·25
June	61,579	3·44	8·79	12·23
July	57,465	3·53	8·69	12·22
August	52,444	3·60	8·68	12·28
September	55,340	3·68	8·76	12·44
October	59,828	3·80	8·82	12·62
November	59,471	3·81	8·81	12·62
December	53,269	3·75	8·78	12·53
Total averages	693,315	3·61	8·77	12·38

Table 3.18—Monthly average composition of milk: Food and Drugs Act samples, 1910–1934

Month	No. of samples	Fat per cent	Solids-not-fat per cent	Total solids per cent
January	27,687	3·63	8·82	12·45
February	29,324	3·58	8·81	12·39
March	31,158	3·57	8·79	12·36
April	27,734	3·56	8·78	12·34
May	30,410	3·52	8·84	12·36
June	29,370	3·46	8·87	12·33
July	26,954	3·54	8·77	12·31
August	23,568	3·62	8·75	12·37
September	28,774	3·72	8·83	12·55
October	30,890	3·82	8·88	12·70
November	30,038	3·81	8·86	12·37
December	25,777	3·73	8·84	12·57
Total averages	341,684	3·63	8·82	12·45

very slight, confirming the opinion that the stage of lactation has a much greater effect than the change of food.

The samples dealt with on pp. 74 and 75 have been arranged and averaged in accordance with the month in which they were taken. They are divided

Table 3.19—Monthly average composition of milk: commercial samples, 1934

Month	No. of samples	Fat per cent	Solids-not-fat per cent	Total solids per cent
January	27,539	3·68	8·75	12·43
February	26,307	3·57	8·75	12·32
March	29,312	3·52	8·72	12·24
April	29,501	3·48	8·69	12·17
May	34,947	3·42	8·74	12·16
June	32,209	3·42	8·72	12·14
July	30,511	3·51	8·61	12·12
August	28,876	3·58	8·62	12·20
September	26,566	3·63	8·69	12·32
October	28,938	3·78	8·74	12·52
November	29,433	3·81	8·77	12·58
December	27,492	3·77	8·73	12·50
Total averages	351,631	3·59	8·71	12·30

into three tables. Table 3.17 deals with all the samples, Table 3.18 with the Food and Drugs Act samples, whilst Table 3.19 deals with the samples submitted by Messrs. Cadbury and by United Dairies Ltd. The fat is at its highest in the autumn and at its lowest in May and June. The solids-not-fat are at their lowest in July or August. They are also low in April, thus confirming the statement, which was apparently first made by G. Cecil Jones, that there is a drop in the solids-not-fat during this month, the figure returning to normal in May.

Davis (1952a) has emphasised that whereas in 1895–1900 there was only a slight fall in s.n.f. in late summer, there is now a marked fall in late winter and early spring. The factors responsible are probably shortage in feeding stuffs and the increase in winter milk production. See also Rowland (1948, 1951a).

(k) The stage of lactation

The stage of lactation has some influence upon the composition of milk, particularly upon the fat content.

Tocher (1925) found that the percentages of both fat and solids-not-fat fell during the first three or four months of the lactation period, afterwards rising to a higher percentage than they were in the first fortnight. The actual amount of change in the case of both fat and solids-not-fat is about the same. The percentage of each ingredient falls, on the average, about 0·2 below that in the second week of lactation and then rises to about 0·2 above, i.e. there is a difference of about 0·4 per cent in both fat and solids-not-fat between the lowest and highest points.

Similar figures have been found by Crowther (1905) and by Drakeley (1927) so that even throughout the whole of the lactation period the changes in the composition of the milk are not very great.

Eckles and Shaw (1913) have given the figures obtained from the milk of 12 cows for fourteen stages of the lactation period commencing five days after calving, viz.—

Table 3.20—Composition of milk at different stages of lactation (*Eckles and Shaw*)

Stage of lactation	*Solids-not-fat per cent*	*Fat per cent*
1	8·74	4·00
2	8·41	3·85
3	8·50	3·79
4	8·47	3·77
5	8·53	3·82
6	8·71	3·79
7	8·78	3·83
8	8·85	3·85
9	8·81	3·97
10	9·05	4·11
11	9·24	4·22
12	9·50	4·54
13	9·57	4·66
14	10·21	5·08

These results and others, such as those of Ragsdale and Turner (1922), Becker and Arnold (1935), and Crowther and Rushton (1911), show that both the fat and the solids-not-fat tend to decrease slightly during the second, third and fourth months, and that after this they gradually increase to the end of the lactation period. It is, of course, well known that the yield of milk progressively decreases as lactation continues.

Such changes as occur throughout the normal lactation period must not be confused with those in the composition of the secretion from the udder which take place immediately after parturition, when the colostrum, as the first secretion is called, passes in the course of a few days to milk proper. The following figures for the composition of colostrum have been given by Walker in Offerton Bulletin No. 3—they can be regarded as showing the type of change which takes place, i.e. a very considerable reduction in the amount of proteins and mineral matter present, and an increase in the amount of lactose.

Table 3.21—Composition of colostrum

	Cow No. 1, 1*st day*			*Cow No.* 2, 1*st day*			*Mixed*				
	a.m.	noon	p.m.	a.m.	noon	p.m.	2nd Day	3rd Day	4th Day	5th Day	6th Day
Total solids	21·54	20·54	20·24	22·30	17·68	16·32	—	—	—	—	—
Proteins	14·85	13·05	10·37	17·50	12·14	10·34	7·40	5·87	5·23	4·53	4·27
Lactose	1·81	2·37	2·80	1·64	3·22	3·81	3·75	4·40	4·87	4·25	4·28
Ash ..	1·13	1·16	1·24	0·48	0·96	0·68	—	—	—	—	—

	Cow No. 3			3rd Day	4th Day	5th Day	6th Day	7th Day
	1st Day E.	2nd Day M.	2nd Day E.	Mixed morning and evening				
Total solids	26·52	19·98	17·52	—	—	—	—	—
Proteins	17·41	4·98	8·36	6·25	5·10	4·98	4·27	4·21
Lactose	1·76	2·30	3·20	3·57	2·69	4·03	3·53	3·87
Ash	0·86	0·68	0·90	—	—	—	—	—

Anantakrishnan *et al.* (1946a) found that colostrum fat contained more oleic and less of the volatile fatty acids than normal milk fat. The fat became normal at about the tenth day after calving.

Smith (1948) has compiled a review of the immune proteins in colostrum and emphasised the significance of the higher protein content (up to 25 per cent) and the different protein distribution. Colostrum in general is richer in immune bodies than the blood of the mother. Parrish *et al.* (1948) have published data showing the gradual fall in total protein and casein, and especially in albumin and globulin as the colostrum changes to normal milk.

Vanlandingham *et al.* (1949) have given nitrogen distribution figures for colostrum and prepartum milk.

Sarkar *et al.* (1949) have reported amino acid contents of colostrum and milk protein as follows (Table 3.22)—

Table 3.22—Amino acids as percentage of protein

	1 *hr.*	24 *hr.*	60 *days*	90 *days*
Arginine	5·3	5·0	3·8	4·0
Histidine	2·8	2·9	2·8	3·2
Isoleucine	5·9	6·3	7·0	7·1
Leucine	9·7	8·8	10·3	10·0
Lysine	7·6	7·2	7·8	6·8
Methionine	1·7	1·7	2·4	1·8
Phenylalanine	4·6	4·5	5·3	4·5
Threonine	6·9	6·0	5·0	4·5
Tryptophane	1·8	2·3	1·5	1·7
Valine	8·8	8·3	7·3	7·5

(l) and (m) The frequency and interval of milking

The effect of the frequency of milking can be usefully considered at the same time as that of variation of the intervals between milkings. Both these factors may have a profound influence on the amount of fat in the milk, but in

Table 3.23—Fat percentage, morning and evening milk

Source	Cows	Morning milk		Evening milk	
		Fat per cent	Interval	Fat per cent	Interval
Offerton Hall	Selected from 36-38 Shorthorns	3·7	14 hours	4·0	10 hours
		3·4	14 ,,	4·1	10 ,,
Seaton Delaval	6 Shorthorns	3·6	13½ ,,	4·1	10½ ,,
Garforth No. 17	Not specified	3·2	14½ ,,	4·5	9½ ,,
,, ,, 44		2·9	14½ ,,	4·2	9½ ,,
,, ,, 50		3·0	14½ ,,	4·1	9½ ,,
,, ,, 60		3·1	14½ ,,	4·3	9½ ,,
Edin. & E. of Scot. Coll. of Agric. No. 15	,, ,,	3·5	13 ,,	3·7	11 ,,
,, ,, 19	,, ,,	3·5	13 ,,	3·9	11 ,,
		3·7	12 ,,	3·4	12 ,,
D. A. Gilchrist	,, ,,	3·2	14 ,,	4·0	10 ,,
Lancs. Farmers' Bulletin No. 5	,, ,,	3·3	14 ,,	4·0	10 ,,
		3·4	14 ,,	4·2	10 ,,
		3·4	14 ,,	4·1	10 ,,

general they have little if any influence upon the solids-not-fat, beyond that which is produced by dilution of the milk with milk-fat.[1]

It is generally agreed that where cows are milked twice a day the evening milk is richer than the morning milk, the larger part of the difference being due to the unequal times of milking. In Table 3.23 a number of results are given, showing the type of difference which is usually found between morning and evening milk.

The following figures for the fat in the milk of individual cows may be taken as typical of average commercial milking, the intervals being 13 hours and 11 hours respectively—

Table 3.24—Morning and evening milk, individual cows

Morning	..	3·7	3·7	3·9	3·7	3·6	3·1	3·2	3·6	3·6		
Evening	..	3·7	3·9	4·1	3·7	3·9	3·4	3·6	3·6	3·3		
Morning	..	3·6	3·0	4·2	3·6	3·8	3·2	3·0	3·7	2·9	3·3	3·3
Evening	..	3·7	3·3	4·1	3·9	4·3	3·6	3·6	4·0	3·4	3·3	3·6
Morning	..	3·8	3·6	3·5	3·4	3·7	3·4	2·9				
Evening	..	3·9	4·0	3·9	4·2	3·9	3·5	3·4				

The effect of alteration in the intervals between milkings is indicated by an experiment described in Offerton Bulletin No. 14, in which two lots each of 5 Shorthorns were milked, one with equal intervals of 12 hours each, and the other with intervals of 14 hours and 10 hours respectively. The results obtained were as follows—

	Lot I Milking at 6 a.m. and 4 p.m.		Lot II Milking at 6 a.m. and 6 p.m.	
Average fat per cent (17 days)	Morning ..	3·6	Morning ..	4·0
	Evening ..	4·3	Evening ..	3·6

Collins computes that a change of one hour in the milking period will alter the percentage of fat in the milk by 0·2; when the interval is longer the amount of fat is less and vice versa. This computation is, of course, only approximate and implies that no other factors are operating at the same time.

Campbell (1931 and 1932) has found that, as far as winter conditions are concerned, night itself, or factors operating at night, tend to produce a greater quantity of milk of lower fat content.

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[1] This influence may be explained by following the effect of the addition of fat to fat-free milk. Let us assume a milk entirely devoid of fat which contains 9·0 per cent of solids-not-fat. If then 10 per cent of fat be incorporated in this milk there will be present 90 parts of fat-free milk, containing 9·0 per cent of solids-not-fat and 10 parts of fat. In the mixture consisting of fat-free milk and fat, there will not be 9·0 per cent of solids-not-fat, but $\frac{9\cdot 0 \times 90}{100}$ per cent, or 8·1 per cent. When the fat in a milk increases from 0 to 10 per cent, the solids-not-fat are decreased in the proportion of 9·0 to 8·1. In the same way, when the variations in fat are much less, proportional reductions in the solids-not-fat take place. As a rough indication it may be assumed that, in the same milk, if one per cent of the fat be removed, the solids-not-fat will be apparently increased by 0·1 per cent.

Heavy yielding cows in fresh lactation are sometimes milked three times a day, although the practice appears to be dying out. The effect of three-times-a-day milking has been studied by several investigators.

Gilchrist (*Dairy Investigations*, p. 3) found the noon milk to be richer in fat than the evening, and that both were better than the morning milk. This investigator found for 12 cows milked at 5 a.m., 1 p.m. and 6 p.m. the following figures—

Table 3.25—Composition of milk: three-times-a-day milking

	Morning	*Noon*	*Evening*
Fat per cent	3·36	4·26	4·16
Solids-not-fat per cent	8·94	9·00	9·21

Similar results have been obtained by C. Brymer Jones, Offerton Bulletin No. 1. Lots of 10 cows were milked in the morning, at noon, and in the evening, the percentages of fat being found as given in the following table—

Table 3.26—Percentage of fat: three-times-a-day milking

Lot	a.m.	Noon	p.m.
1	2·96	3·62	4·29
2	2·51	3·72	4·50
1	2·97	3·94	3·90
2	2·57	3·69	3·63
1	3·23	3·83	3·70
2	3·03	3·75	3·68

Bartlett (1934) states that there is little to suggest a decrease in non-fatty-solids as milking progresses if calculations are made on the fat-free milk, whilst there is no difference between morning and evening milk if the calculation is made on the same basis. He further states that the first-drawn morning milk tends to be very poor in fat and the last-drawn exceptionally rich, whilst the evening milk tends to show less variation in fat between the first- and last-drawn portions. The difference in fat content between the first- and last-drawn milk is greater with high-yielding cows than with those giving low yield. Gravity does not completely account for the " strippings " being rich in fat, as a goat milked on its back, and having its udder massaged, gave " strippings " very rich in fat. (Compare Section (*n*).)

It is well recognised that interrupted milking results in a considerable increase in the total protein content of milk. Dicks *et al*. (1951) have reported that while the casein content did not change, the globulin, albumin and non-protein nitrogen of milk were significantly increased.

(n) The personnel factor of milkers

The percentage of fat in the milk may be considerably altered by the inefficiency of the milker, and it must also be remembered that while some animals do not appear to be influenced by alterations in the personnel of the milkers, other animals show their objection to being milked by strangers and hold up their milk.

It is a well-known fact that the milk first drawn from a cow (" fore-milk ") differs very materially in its fat content from that last drawn (" strippings "). The " fore-milk " is very poor in fat while the strippings are rich in fat, a steady increase in the amount of fat present in the milk taking place as the milking proceeds. The following figures obtained in the Lancashire County Laboratory indicate the amount of alteration which is likely to take place under the conditions mentioned.

Table 3.27—Percentage of fat: fractional milkings

Source	1st portion	2nd portion	3rd portion
Evening milk, 1 cow ..	2·0	3·7	5·2
Evening milk, 1 cow ..	2·1	3·6	6·1
Morning milk, 5 cows	3·0	—	5·1
Evening milk, 1 cow ..	2·3	3·0	4·6
Evening milk, 1 cow ..	2·3	3·5	4·9
Morning milk, 5 cows	—	3·3	8·2

(o) Sexual excitement

There does not appear to be any generally recognised rule as to how the composition of the milk of cows varies during the season of oestrum or heat. Mackintosh observes that " some give a decreased yield with a higher fat percentage; others a decreased yield with a lower fat percentage, followed in both cases by a material increase in the yield for one or two milkings. Others, again, show very little variation in yield or percentage of fat." The writers, however, basing their opinion on many of the published results, consider that the cases where there is an increase in the fat content far outnumber those in which there is a decrease.

(p) Variations in the yield of milk

Where the milk yield of a particular cow varies there is often a variation in the fat content, a smaller yield having a higher percentage of fat, and a larger yield having a low percentage of fat. This does not hold good for animals habitually giving small or large yields, for the milk of cows with a small yield is not necessarily richer in fat or total solids than that of cows with a large yield; in fact the opposite may be the case.

Likely day-to-day variations in fat content

It might be considered that since the amount of fat in milk may be altered, even if only to a small extent, by any of some fifteen factors, it would be impossible to forecast the changes from day to day in the fat content of the milk of any particular animals. More careful consideration of the factors involved will, however, show that such is not necessarily the case. In the first place, many of the factors liable to cause variation do not, of course, operate when the same cow or the same herd is under consideration. Thus the number of animals, the breed, the individuality, the age, the climate, the season, the stage of lactation, and the conditions of management will be constant, or practically so, for any given herd within a short time, whilst with the other factors where some variation may be expected, or at least may be possible, the change will, in a herd, be gradual and not sudden, so that, e.g. appeal-to-cow samples will not be sufficiently altered to invalidate their use as comparison samples. When the whole herd is under consideration, it is generally admitted that fluctuations in the composition of the milk of individual animals will tend to neutralise one another, and consequently that the variations found for the milk of the whole herd will be considerably less than that for any one cow.

There is one factor which may, but not necessarily will, cause a sudden change in the composition of the milk. This is health. Cows that are taken with sudden indisposition may yield milk varying quite widely in its composition, but this possibility is not likely to affect the taking of an appeal sample, as, in the first place, the illness of the cow will be noticed and allowed for, and, secondly, the same illness is unlikely to attack all the animals in a herd at the same time, so that taking the milk of the whole herd into account very little change will be noticed.

From a consideration, therefore, of the factors which tend to cause variations in the percentage of fat in milk, it would appear that, in general, little variation is actually to be expected. Such is indeed the case, provided of course, that the milking is carried out under similar conditions on each occasion. The point can only be decided by examining the milk of a herd of cows from day to day over a considerable period.

A large number of such day-to-day examinations are available, but it must be emphasised that many of these have been published with the idea of showing how wide the variations may be in certain cases, and do not by any means give a true idea of the average variations likely to be found. Indeed, in certain of the published results the variations are so extraordinary that they are far more easily explained by some error having crept into the methods. When one result is produced which differs from all others in the same series and from almost all other series, and is quite at variance with the experience of the majority of competent investigators, it can only be accepted as final when it is confirmed by duplicate results of two or more skilled analysts working independently.

The available evidence indicates that the possible day-to-day variation in the fat content of the mixed milk of a herd is rather greater than that in the solids-not-fat, although such variation has undoubtedly been exaggerated in various quarters. It would appear that the percentage of fat is altered more quickly by changes in the environment of the cows than is that of the solids-not-fat, but it further appears that, in general, a sudden fall due to very unfavourable conditions is far more probable than a sudden improvement due to more favourable ones. It is extremely unlikely that milk containing less than 3 per cent of fat, even from a small herd, would increase its percentage of fat by more than 0·5 in two days or less, it being assumed, of course, that corresponding milkings are being considered and that the conditions of milking are the same in both cases (since by altering the time and taking only part of the yield, almost any percentage of fat from one to ten can be obtained). The amount of possible alteration depends to a certain extent on the amount of fat present, for more variation is to be expected in a milk containing a large amount of fat than in one containing only a small amount. This again is all to the good, for it means that it is most unlikely that a milk which is considerably under the statutory limit one day will be considerably over it within two or three days. In order to show what, in the opinion of the writers, are likely variations in genuine milks and what variations may possibly be put down to natural causes, the following table (3.28) has been compiled. In the first column is the percentage of fat found in a sample taken under the Sale of Food and Drugs Act, whilst in the second column is the percentage of fat found in the corresponding appeal-to-cow sample. In the third column is placed the decision as to whether a prosecution should be instituted or not. It must not be assumed that in all those cases where prosecution was not advised no suspicions were entertained, such indeed was far from the case in some instances; but it can be assumed that where a prosecution was advised, moral certainty of adulteration existed.

Table 3.28—Comparison of appeal-to-cow samples with samples low in fat

Original sample fat per cent	Appeal-to-cow sample fat per cent	Proceedings advised
2·1	2·4	No
2·6	2·9	No
2·5	3·5	Yes
2·4	2·9	No
2·1	3·0*	No
2·6	4·2	Yes
2·5	3·3	Yes
2·6	3·5	Yes
1·7	3·0	Yes
2·7	3·1	No
2·3†	2·9	Yes
2·3	3·2	Yes
2·4	3·4	Yes
2·4	3·6	Yes
2·3	3·6	Yes
2·0	3·7	Yes

* Individual cow. † Also deficient in solids-not-fat.

The following paragraphs contain some account of the published figures showing daily variations of the fat in the milk of herds of cows.

In Bibby's *Book on Milk* the fat content of the morning milk of 25 Shorthorn cows is given daily for three weeks. In this time the extreme variation found was from 3·8 to 2·9 per cent, the biggest daily variation being 0·7 per cent, from 3·1 to 3·8. It should be noticed, however, that after the 2·9 it did not rise again as high as 3·2 before the end of the experiment, which took place five days later.

In the paper by Lauder and Fagan (1914) the daily percentage of fat in both the morning and evening milks is given for two herds, each containing 10 cows, over a period of 10 weeks. In these two herds a daily variation in the fat of the morning milk of 0·6 per cent occurred on four occasions, but in none of them was the lower percentage less than 3·0 per cent. A daily variation of 0·5 per cent occurred once, whilst a daily variation of 0·4 per cent occurred three times.

In all other cases the daily variation was 0·3 per cent or less. In the case of the evening milk the greatest daily variation was 0·5 per cent, which occurred twice. A variation of 0·4 per cent occurred six times, whilst a variation of 0·3 per cent occurred twice.

Elsdon and Stubbs (1930a) examined the milk of two herds daily. The larger herd contained 39 to 45 non-pedigree Shorthorns, whilst the smaller herd, which also consisted of non-pedigree Shorthorns, contained 11 to 14 cows. The larger herd was sampled both morning and evening for 134 consecutive days, and the smaller herd was sampled for 50 consecutive days about a year after the work on the larger herd. Both sets of experiments covered the periods when the cows were brought in at night and when they were kept in entirely. The maximum daily variation in the fat content (increase) was 0·6 per cent, which occurred on two occasions in the morning milk of the larger herd. The daily increases of more than 0·2 per cent are set out in Table 3.29.

Table 3.29—Daily variation of fat in milk of herds

Increase in one day	Number of times increase occurred			
	Larger herd		Smaller herd	
	Morning	Evening	Morning	Evening
0·6	2	0	1	0
0·5	2	2	1	0
0·4	5	5	2	2
0·3	6	4	2	5

The following example will serve to illustrate the difficulty which may be experienced in attempting to assign variations in the composition of milk to any particular governing factor. During the eight months January to August of the year 1940, 611 samples of milk were examined under the Food and Drugs Act in the Salford City Laboratory, and of these 51 were found to have solids-not-fat below 8·5 per cent, although they had normal Hortvet freezing-points, indicating that they were of naturally poor quality. The figures for the corresponding period of 1939 were 670 samples of milk, of which 10 were poor in solids-not-fat but had normal freezing-points, and for the same period of 1938, 592 samples of milk, of which 9 were poor in solids-not-fat but had normal freezing-points. The increase in the number of abnormal samples during the period of the year 1940 under discussion is very striking, and during conversation with other Public Analysts it was found that this was by no means confined to the samples examined in Salford. Of the 51 abnormal samples examined in the first eight months of the year 1940, 36 were taken during the months of January to April inclusive; although, therefore, approximately two-thirds were obtained during the winter months, during which the weather was unusually severe, the remaining abnormal samples examined during the early summer months were still materially greater in number than the whole of the abnormal samples found in either of the two preceding years.

In addition to the 51 samples already mentioned, six "appeal-to-cow" samples were taken in connection with certain of the samples in order to make quite sure that there had been no tampering with the milk and that the abnormal results were really due to natural causes. The figures for these are given below, and they serve to illustrate the degree of variation found—

Table 3.30—"Appeal-to-cow" samples of low solids-not-fat and normal freezing point

No.	Total solids per cent	Fat per cent	Solids-not-fat per cent	Freezing-point (Hortvet)
A 5924	11·69	3·70	7·99	—0·553° C
A 5925	11·57	3·63	7·94	—0·563° C
A 6454	10·01	2·45	7·56	—0·538° C
A 6455	11·04	3·10	7·94	—0·543° C
A 6456	11·25	3·05	8·20	—0·548° C
A 6457	11·06	2·90	8·16	—0·553° C

The first two samples represent the mixed evening milk of a herd of 14 cows, the total yield being 23 gallons. The cattle were pedigree Friesian stock, the farmer had ample supplies of feeding-stuffs, and he could not offer any explanation for the poor quality of the milk.

The four remaining samples were taken at another farm. Nos. A 6454 and A 6455 are samples of the evening milk of two individual cows which were related to one another. The yield at this milking was 3¾ gallons from the first cow and 3 gallons from the second cow. Samples Nos. A 6456 and A 6457 represent the mixed evening milk of the herd of 13 cows and include representative portions of the milk of the two individual cows mentioned above. The total milk yield was 17 gallons, of which 6¾ gallons was therefore due to two cows. In this case also the herd was Friesian stock and, although the farmer had been short of feeding-stuffs earlier in the year, for some time prior to the date of sampling he had had ample supplies.

Although "appeal-to-cow" samples were taken in these two instances and the cows concerned found to be of Friesian stock, which often yield milk of poorer quality than many other breeds, this should be taken with a certain amount of reserve, as enquiries by the sampling officer with regard to other abnormal samples showed that herds of mixed breeds were also affected. In any event, the question of breed would not explain the fact that abnormal samples had been fewer in number in previous years.

With regard to feeding-stuffs, there is no doubt that owing to the war these were difficult to obtain, and many farmers had experienced a shortage, but in the two cases quoted this factor could have little if any significance.

The weather was a factor which might have been of some significance. Meteorological reports showed that January, 1940, was the coldest month locally since 1895, and the winter generally was extremely severe. On the other hand, June was the sunniest month for at least the last 70 years. The fact that many abnormal samples were obtained, in both the winter and early summer months of the period under consideration, would suggest that change of temperature might be a contributory factor.

Summarising these findings, it appears that the weather might be responsible for many of the abnormal results obtained; in other cases this factor, coupled with the breed and individuality of the cows and a shortage of suitable feeding-stuffs, might have a combined effect. During the investigation of these samples no evidence was obtained that these factors, either singly or in combination, caused any *sudden* alteration in the composition of milk.

Recent investigations

About half-a-million results have been tabulated by H. Burr (1939). He was unable to find any correlation between solids-not-fat and feeding succulent foods in winter, type of soil, height above sea-level or size of herd. Good condition of the cows, low incidence of mastitis and the feeding of supplementary foods in summer were conducive to high solids-not-fat. Rowland (1944) has given details of an investigation at a number of creameries during March–April 1942. The mean s.n.f. was 8·51 and 48 per cent of the samples were below 8·5 per cent. In May the mean was 8·68 and only 25 per cent were below 8·5 per cent. In 1943 the mean was 8·45 per cent with 55 per cent of the samples below 8·5 per cent from January to April. It is suggested that reduced feeding levels in this area accounted for the low s.n.f. in winter and the rise when young grass became available. A similar decline in solids-not-fat has been experienced in France. Eyrard *et al.* (1946) have shown that the s.n.f. of milk supplied to Paris has fallen steadily over the period 1940–45. The maximum values occurred in early winter and the minimum in August. O'Loughlin and Ryan (1944) have reported that in Eire in 1942, 3·8, 2·4 and 0·4 per cent of herd samples fell below 8·5, 8·3 and 8·0 per cent s.n.f. respectively. The range of freezing points was –0·536° to –0·564 °C and values calculated by Beckel's formula (p. 139) agreed closely with the experimentally determined values.

Provan (1949) records a fall in the s.n.f. of bulk milk from 8·9 per cent in 1923 to 8·6 per cent in 1946. He estimates that it is now possible for bulk Friesian milk to be below the legal standard for 18 weeks in the year, the period for Shorthorn milk being 11 weeks and for Ayrshire 10 weeks. Fat values have altered little and the average values for breed milks in 1945–47 were: Jersey and Guernsey 4·41 and 8·93, Ayrshire 3·72 and 8·73, Shorthorn 3·65 and 8·68, and Friesian 3·45 and 8·58 per cent for fat and s.n.f. respectively.

Variations in bulk milk have been recorded by Rees (1949) as follows—

	May	*Aug.*	*Nov.*	*Feb.*	*May*
Fat, per cent	4·7	4·5	4·3	4·4	4·7
s.n.f., per cent	9·1	8·7	9·2	8·5	9·0
Freezing point, °C ..	—	–0·532	–0·552	–0·527	–0·545

This period included prolonged dry weather in late summer.

Relation between fat and solids-not-fat

Considering average values for a large number of samples, there is a marked correlation between fat and solids-not-fat percentages, the fat increasing by about 0·4 per cent for every 0·1 per cent increase in solids-not-fat.

Richardson and Folger (1950), have recently studied this subject and drawn attention to the lack of agreement between workers, some finding a linear relationship and some not. The following equations have been given for the linear relationship—

s.n.f. = 0·4 per cent fat + 7·07 (Jacobson, 1936).
= 0·346 per cent fat + 7·597 (Marquardt, 1934).
= 0·3846 per cent fat + 7·6736 (Overman *et al.*, 1939).
= 0·7481 (7·8903 + per cent fat) (Kahlenberg and Voris, 1931).

Richardson and Folger find that the relationship is linear over only a restricted fat range. As might be expected, they find no consistent relationship for mastitis-positive cows. They suggest the following equations—

Fat range	*Normal milk*	*When 20–25 per cent cows mastitis positive*
2·75—3·30	s.n.f. = 0·3151 fat + 7·3672	0·35 fat + 7·18
3·30—4·25	0·70 fat + 6·10	0·7041 fat + 6·0056
4·25—5·85	0·3846 fat + 7·44	
4·25—6·00		0·4007 fat + 7·2936
5·90—6·75	0·2457 fat + 8·2340	
6·00—6·75		0·0959 fat + 9·1248

They also consider that a hereditary factor from the sire is involved in the secretion of solids-not-fat.

Mork (1947) has deduced the following relationship from analyses of 113 samples of Norwegian milk—

Protein .. .. = 1·73 + 0·38 fat (± 0·26)
Lactose .. .. = 5·33 — 0·11 fat (± 0·26)
Ash .. .. .. = 0·64 + 0·02 fat (± 0·04)
Total solids .. .. = 7·7 + 1·29 fat

General

For general surveys of this subject see Board of Agriculture (1901), Min. Agriculture (1932), Hanley (1936), and the reviews on dairy chemistry in the *Journal of Dairy Research.*

CHAPTER 4

MILK OF MAMMALS OTHER THAN THE COW

Classification

Broadly speaking, the milk of all mammals may be divided into classes as under—

(1) Milks forming hard curds with rennet. This class includes the milk of the ewe, buffalo, goat, and cow.

(2) Milks forming a very soft, or no curd with rennet. Included in this class are human milk, and those of the ass and mare.

This difference can be correlated with differences in pH, calcium/phosphate and casein/albumin ratios. Usually the lower the pH, the higher the calcium and casein and the firmer the clot given by rennet. Addition of citrate, heating and enzymic digestion all reduce the firmness and particle-size of rennet curd (Davis, 1953).

The composition of the milk of all mammals, on the whole, resembles that of cows' milk, i.e. they all contain fat in the form of globules, sugar, proteins, and mineral matter. Marked differences, however, occur in the composition of these bodies.

Comparison of the fat of milk of different animals

The following table gives the results obtained by Pizzi with regard to size of globules—

Table 4.1—Size of fat globules in mammalian milks

Name of mammal	Relative number of globules of the sizes named							
	0·0127 mm	0·0109 mm	0·0090 mm	0·0072 mm	0·0054 mm	0·0033 mm	0·0018 mm	0·0009 mm
Woman	—	—	many	many	medium	few	v. few	v. few
Ewe	—	few	—	medium	,,	medium	few	,,
Goat	v. few	v. few	few	few	,,	,,	medium	,,
Cow	—	—	medium	many	,,	,,	v. few	,,
Rabbit*	many	many	few	few	v. few	v. few	,,	,,
Ass	—	v. few	v. few	many	medium	medium	v. many	,,
Mare	—	,,	medium	medium	few	many	many	,,
Sow	—	—	v. few	v. few	v. few	medium	v. many	many
Bitch	—	—	many	many	medium	,,	v. few	v. few
Cat	—	—	few	few	,,	few	,,	,,
Mouse*	many	many	,,	,,	,,	v. few	,,	,,

* The milk of the rabbit and mouse contained globules up to 0·0181 mm in diameter.

Human milk

Human milk is one of the few which form granular and flocculent precipitates rather than coagulate into a hard mass. Human milk also differs rather sharply from others in its general composition. It has usually a chalky-white, somewhat watery appearance; some specimens, usually those high in proteins, have a marked yellowish tint. The fat globules, according to Pizzi, vary in size from 0·009 mm to 0·0009 mm. Carter and Richmond have observed that they are, on the whole, smaller than those of cows' milk. The taste is rarely, if ever, sweet, but rather saline. The reaction to litmus paper is almost always alkaline. The acidity is about 3·0°, and, in Richmond's experience, has varied from 1·3° to 5·5°, while Bosworth gives 3° to 6°.

Composition

Human milk appears to be more variable in its composition than that of the cow. This is probably due to the fact that, while the cow is forced to adopt ordered habits and leads a life which is very regular, the many occupations and duties of women do not permit of this.

Table 4.2 gives the mean composition of human milk according to observers quoted by Richmond.

Table 4.2—Mean composition of human milk (per cent)

Observer	Water	Fat	Sugar	Proteins	Ash
Leeds	86·69	4·16	6·95	2·02	0·22
Pfeiffer	88·22	3·11	6·3	1·94	0·19
Luff	88·51	2·41	6·39	2·35	0·34
Johanssen	—	3·21	4·67	1·1	—
Carter and Richmond ..	88·04	3·07	6·59	1·97	0·26
Lehmann	87·3	3·4	6·4	1·7	0·2
Camerer and Söldner ..	88·07	3·24	6·33	1·69	0·24
Szilasi	87·24	3·38	6·97	2·2	0·2
Backhaus	87·41	4·02	6·71	1·62	0·25
Elsdon	88·3	3·11	7·18	1·19	0·21
Gottlieb	87·52	3·38	7·51	1·17	0·27
Bosworth	—	3·3	6·5	1·5	—
Richmond	88·53	3·23	6·75	1·25	0·23
COLOSTRUM—					
Pfeiffer	85·75	2·38	3·39	8·6	0·37
Lajoux, 1st day	83·45	3·5	4·59	8·08	0·46
,, 2½ days	89·3	1·45	5·91	3·05	0·29

Elsdon (1928) reported in 1928 the analyses of 529 samples of human milk taken from eighty-six different subjects. The average of total solids obtained from the milk of each subject varied from 10·1 to 13·4, the average for all samples being 12·07 per cent. The corresponding figures for solids-not-fat were from 7·8 to 9·9 with an average of 8·80, and for fat from 1·0 to 5·1 with an average of 3·27. The individual samples giving the highest and lowest amounts of fat yielded 10·2 per cent and 0·4 per cent, the corresponding figures for solids-not-fat being 17·3 per cent and 3·8 per cent. Gardner and Fox (1925) reported average figures of 8·7 per cent for solids-not-fat and 3·7 per cent for fat. Myers (1929) found the average of eighty-six samples to be 3·95 per cent for fat and 11·7 per cent for solids-not-fat. Dart (1926) found as averages for milk of Australian women $\Delta = 0{\cdot}583°$; lactose, 6·45 per cent; proteins, 1·40 per cent; ash, 0·19 per cent; fat, 5·3 per cent. Bunce states that there is relatively little difference between the composition of human milk in Burma, India, Anglo-India and Europe. Kayser (1939) reports the composition of the milk of 100 donors in Erfurt. The average percentage of fat was 3·2 (extremes, 0·6 to 9·7) and of solids-not-fat 8·7 (extremes, 8·0 to 9·5). Jerlov (1931) found from 0·035 to 0·125 per cent of citric acid. Sydow (1947) has emphasised the errors likely to influence data on the fat content of human milk. From 1,004 samples he obtained an average of 3·1 per cent. Gölz (1940) has found the fat in human milk to vary from 0·5 to 10 per cent (mode 3·5). The effects of the usual factors are discussed in this paper. Tarján *et al.* (1950) report the following average values in Budapest: specific gravity 1·023, total solids 10·9, fat 3·6, sugar 5·8, protein 1·3 and ash 0·15 per cent. Stoldt (1949) gives the following range of values for human milk (48 samples): fat 1·6–5·4, ash 0·13–0·26 and serum *n* at 17·5° C 44–51.

Chanda *et al.* (1951) have given analyses for human milk obtained in Aberdeen. The s.n.f. value was initially about 11·0 but later varied from 9·0 to 10·0, and the percentage fat ranged from 2·5 to 3·9. This was much more variable than the protein which was low immediately after birth but rose to about 2·0 per cent on the third day and remained at about that level.

These authors note that the N : P ratio was 20 in human milk whereas this is only 6 in cows' milk.

Whittle (1943) reports that freezing points of human milk ranged from —0·517° to —0·566° C.

A comprehensive report on the nutritive value of human milk has recently been published by Kon and Mawson (1950). This contains many analytical data.

Variation in composition with period of lactation

A number of series of analyses have been published in which the variation in composition with the stage of lactation has been noted. The consensus of opinion is that the percentage of fat increases as the lactation advances, whilst there is a diminution in the amount of protein and ash. There is some evidence that the amount of sugar tends to increase. Brown *et al.* (1932) state that they found that, whilst the amount of fat decreased at first, it regained its early high value late in lactation. Elsdon (1928) confirmed these conclusions in the series already referred to, the results being obtained as shown in Table 4.3.

Variation of constituents

The following maxima and minima have been found (per cent)—

Fat	9·05 (Pfeiffer)	0·47 (Carter & Richmond)
Sugar	8·89 (C. and R.)	4·22 (Pfeiffer)
Protein	5·56 (Pfeiffer)	0·85 (Leeds)
Ash	0·50 (C. and R.)	0·09 (Pfeiffer)

A number of observers have shown that the composition of the milk given by the two breasts is almost identical, and that the composition of the milk secreted is not much altered by suckling.

Table 4.3—Variation of human milk during lactation

(*A*) *Proteins and ash*

	Proteins per cent	*Ash per cent*
1 day	2·20	0·37
2 days	2·04	0·35
3 ,,	2·11	0·35
4 ,,	1·98	0·29
5 ,,	2·08	0·31
6 ,,	1·91	0·33
7 ,,	1·76	0·28
8 ,,	2·25	0·24
9–12 days	1·77	0·27
13–20 ,,	1·60	0·28
21–50 ,,	1·26	0·27
50 days to 9 months	1·23	0·20
AVERAGE	1·81 for 85 samples.	0·29 for 117 samples.

(*B*) *Fat and solids-not-fat*

Period of lactation	*No. of samples*	*Fat per cent*	*Solids-not-fat per cent*
Up to 7 days	223	2·86	9·01
8 to 28 days	280	3·58	8·58
1 to 9 months	26	3·44	8·48

(*C*) *Average values*

Period of lactation	*Proteins per cent*	*Lactose per cent*	*Ash per cent*
1 to 7 days	2·1	6·8	0·30
8 to 28 days	1·6	6·8	0·25
1 to 9 months	1·3	6·9	0·22

The composition of the fat

The main difference between the fat of human milk and that of cows' milk s that the former contains only a trace of volatile fatty acids. Reichert figures of 1·4 to 3·4 have been recorded, together with Polenske values of 1·9 to 2·2 and Kirschner values of 1·9 to 2·0. Borsarelli (1933) affirmed that the period of lactation does not affect these values, but Polonovski (1933) stated that feeding glucose increases the amount of volatile acids.

Bosworth (1934) found the fat to consist of a relatively large proportion of dodecenoic acid. The other acids include tetra- and hexadecenoic, oleic, and linoleic—probably unsaturated acids with more than 18 carbon atoms—and two highly unsaturated acids of the arachidonic type.

Baldwin and Longenecker (1944) find that human colostrum fat contains

6·1 per cent of phospholipids as against 0·5 per cent in ordinary human milk. About half the fatty acids were saturated and the quantity of volatile acids was very small. Palmitic constituted about one-quarter of the total and oleic over one-third. Human milk contains more hexadecenoic and octadecadienoic acids than cows' milk.

The sugar of human milk

There is considerable doubt as to whether the sugar of human milk is entirely lactose. Polonovski and Lespagnol (1931, 1932 and 1933) claimed to have isolated two new sugars which they have named " gynolactose " and " allolactose ". These may be modifications of lactose, as on hydrolysis they yielded glucose and galactose, but their exact nature does not appear to have been elucidated.

The proteins of human milk

The proteins differ from those of cows' milk in not giving a curd with rennet, and in giving a much finer precipitate with acids. By the addition of calcium phosphate they can be made to approach much more nearly in behaviour to those of cows' milk. The proteins of human milk are not precipitated by copper sulphate from a solution neutral to phenolphthalein, but require a further addition of alkali; the precipitate thus obtained yields a black ash, while the proteins of cows' milk precipitated from a neutral solution leave a green ash.

The casein of human milk, though closely related, differs from that of cows' milk; it is not curdled by rennet, does not exist in combination with calcium phosphate, and is thrown down by acids in a finely-divided state, though Engel found that if 10 g of human milk are mixed with 50 ml of water and 6 to 8 ml of 0·1 N acetic acid, kept at 0° C for two or three hours, and then warmed to 40° C with frequent stirring, the casein separates easily. Dolgrel advised the addition of salts to make the casein flake. Kolbrak considered human casein less acid, and found that on continued precipitation with acid and solution in alkali it became more and more like cows' casein. Lehmann and Hempel found 1·09 per cent of sulphur and 3·2 per cent of ash in the casein of human milk separated by a porous plate, as against 0·72 and 6·47 respectively for cows' milk, and Wroblewski confirmed a higher percentage of sulphur. Sikes found the lime and phosphorus low. Abderhalden, with Schittenhelm and Langstein, found that though biological tests show that the casein of human milk is not the same as that of cows' milk, the proportions of amino-acids are very similar, and Tangl and Czókâs confirm this.

Bosworth and Gibbin state that, when purified, the casein of human milk is identical with that of cows' milk.

M. Ocampo (1933) found that for Philippine human milk 14·9 per cent of the nitrogen exists as casein, 44·8 per cent as albumin, and 16·9 per cent as globulin.

The ash of human milk

Harrington and Kinnicutt give the mean composition of the ash as shown in Table 4.4.

Table 4.4—Ash of human milk

	Per cent
Uncombined carbon	0·71
Chlorine, Cl	20·11
Sulphurous acid, SO_2	4·38
Phosphoric acid, P_2O_5	10·73
Silica, SiO_2	0·70
Carbonic acid, CO_2	7·97
Iron oxide and alumina, $(FeAl)_2O_3$	0·40
Lime	15·69
Magnesia	1·92
Potash	29·84
Soda	12·39
	104·84
Less Oxygen ≡ Chlorine	4·53
	100·31

The presence of citric acid has been established, and its amount is about 0·1 per cent.

The analysis of human milk

The methods of analysis used for human milk are exactly the same as for cows' milk. Total solids are usually determined on 5 g, proteins on 5 or 10 g, ash on the total solids, fat by the Gerber or other standard process, and lactose is usually calculated by difference.

The quantity of sample available for analysis is often very limited, and it may be necessary to take smaller amounts for some of the determinations; in this connection the Gerber process can be modified by using a 5·5 ml pipette instead of an 11 ml pipette, pipetting 5·5 ml of sample followed by 5·5 ml of water into the butyrometer tube, and then carrying out the test as usual. The reading obtained is, of course, multiplied by 2 to give the percentage of fat in the sample.

A number of useful papers on human milk have been published in Vol. 5 of the *British Medical Bulletin*. See Kon (1947) (cows' milk as human food); Platt and Moncrieff (1947) (nutritional comparison of human and cows' milk for infant feeding); Mackintosh (1947) (breast milk banks); Marrack (1947) (antibodies in milk); and Burn (1947) (execretion of drugs in milk).

Apes' milk

Schumacher examined the milk produced by an orang-utang which was nine years old and which had been lactating for two years. The total solids amounted to 11·47 per cent, and it was found to resemble cows' milk rather than human milk. The following constituents were determined: fat, 3·5 per cent; casein, 1·05 per cent; albumin, 0·37 per cent; sugar, 6·02 per cent; non-protein nitrogen, 0·03 per cent; ash, 0·24 per cent; and chlorine, 0·09 per cent.

Asses' milk

Asses' milk is similar in character to human and mares' milk in that it coagulates into small granular and flocculent masses, whilst with that of the ruminants the coagulation takes place into a hard mass. With animals other than the ruminants there is usually a larger percentage of sugar and a smaller percentage of proteins, each of which may have a somewhat different constitution.

Richmond (1896) gave the mean composition as: total solids, 10·23 per cent; fat, 1·18 per cent; sugar, 6·86 per cent; proteins, 1·74 per cent; and ash, 0·45 per cent; whilst Schlossmann (1898) examined 16 samples of asses' milk and found the specific gravity to vary from 1·031 to 1·036. The average content of total solids found was 11·15 per cent, of ash 0·40 per cent, and of sugar 4·94 per cent. The fat varied from 0·15 to 0·60 per cent and the proteins from 1·40 to 1·72 per cent.

Wagner (1907) made 392 determinations of the milk from 30 animals comprising one herd. The average fat over a period of five years was 0·125 per cent, the annual averages being 0·23, 0·14, 0·10, 0·11, and 0·10 per cent, the extremes recorded being 0·00 per cent and 0·70 per cent.

It appears that the average composition of asses' milk is of the order of that given in the table below. There is reason to believe that the variations found between the milk of different animals are likely to be of the same order as those found for the milk of cows; the number of published results is very much less. Similar remarks can be made, to a greater or lesser extent, with regard to the milk of all other animals.

Table 4.5—Average composition of asses' milk

	Per cent
Total solids	9·95
Fat	1·0
Sugar	6·8
Proteins	1·7
Ash	0·45

The Reichert value of the fat is only about half that of cows' butter fat.

Richmond has prepared the sugar and finds that it has a specific rotatory power $[\alpha]_D = 52{\cdot}5°$ (for hydrated sugar), a birotation ratio of 1·6, and corresponds in every particular with milk-sugar.

The milk has a very feeble alkaline reaction to litmus; the acidity to phenolphthalein is about 4·5°; rennet produces a very soft curd after a long time, and acids give a finely-divided precipitate. On boiling, it has a tendency to curdle and deposit flakes (coagulated albumin?). It has a white colour and a sweet taste. The aldehyde figure multiplied by 0·154 gives a close approximation to the proteins (Laxa 1931, and Koschucharoff 1931).

Buffaloes' milk

The milk of the buffalo is of very considerable commercial importance in Egypt and India, where it is sold indiscriminately with cows' milk. As the former tends to be richer, both in fat and solids-not-fat, than the latter, administrative difficulties have been caused by the application of standards suitable for the milk of the one to the milk produced by the other.

Leather (1901) examined the milk of 17 individual buffaloes and also the milk of a herd. The following figures were obtained—

Table 4.6—Buffaloes' milk

Constituent (per cent)	Range	Average	Herd milk
Fat	4·08 to 9·95	7·39	8·09
Proteins	3·24 ,, 4·36	3·88	4·34
Sugar	4·27 ,, 5·12	4·73	4·56
Ash	0·68 ,, 0·82	0·75	0·76
Total solids ..	13·57 ,, 19·84	17·03	17·78
Solids-not-fat ..	9·06 ,, 10·04	9·64	9·69

Similar results have been obtained in Burma by Bunce. Leather gave the Vieth ratio as 12 : 10 : 2.

Hawley (1927) as a result of work on the milk of 33 individual buffaloes, recommended a standard for the Madras Presidency of at least 4·5 per cent of fat and 9·0 per cent of solids-not-fat. Only one of Hawley's samples (8·8 per cent) contained less than 9·0 per cent of solids-not-fat and only two (4·2 per cent and 4·3 per cent) contained less than 4·5 per cent of fat.

The milk of the American buffalo or Bison may not be so rich in fat as that of Indian and Egyptian buffaloes. Shutt (1932) found, in the case of two samples which were somewhat sour, 1·83 and 1·69 per cent of fat and 11·14 and 11·98 per cent of solids-not-fat. The casein was 3·66 per cent and 4·24 per cent and the ash 0·86 per cent and 0·96 per cent.

The following may be regarded as the average composition of the milk of the Indian and Egyptian buffaloes—

Table 4.7—Average composition of buffaloes' milk (per cent)

Total solids	17·1
Fat	7·5
Sugar	4·7
Proteins	4·1
Ash	0·8

Various writers have found the figures given in the following table—

Table 4.8—Percentage composition of buffaloes' milk

Authority	Water	Fat	Milk sugar	Proteins	Ash
Strohmer	81·67	9·02	4·50	3·99	0·77
Fleischmann	82·93	7·46	4·59	4·21	0·81
Pizzi	82·20	7·95	4·75	4·13	0·97
d'Alzac	82·05	7·98	5·18	4·00	0·79
Trillat and Forestier ..	80·72	7·24	5·68	5·38	0·98
Leather	82·96	7·41	4·72	3·91	0·75
Pappel and Richmond ..	84·10	5·56	5·41	3·95	0·85
Pappel and Hogan	82·09	7·95	4·86	4·16	0·78

The milk is always white in colour, and the butter fat prepared from it has only a slight yellowish tinge. Except that it is much richer in fat, and somewhat so in solids-not-fat, it does not differ greatly from cows' milk. The average fat was 7·57, and the variations were from 4·08 to 9·95. Pappel and Richmond for Egyptian buffaloes, and Leather for Indian animals have each pointed out that the ratio of milk-sugar, proteins, and ash is on the average 12 : 10 : 2, as against 13 : 9 : 2 in cows' milk.

Leather found that the freezing point of Indian buffaloes' milk is the same as that of cows' milk. Pappel and Richmond found 0·30 per cent of citric acid in Egyptian milk.

Richmond has calculated the following formula as applicable to buffaloes' milk (see Chapter 6 for explanation of symbols)—

$$T = 0{\cdot}27\,\frac{G}{D} + 1{\cdot}191\ \mathrm{F}.$$

The following notes on the constituents of the milk will show the differences from cows' milk. Bunce (1932) examined the milk of 200 herds; the average results of all samples were—

	No. of samples	*Fat per cent*	*Solids-not-fat per cent*	*Ash per cent*
Morning milk ..	100	6·78	10·16	0·79
Evening milk ..	100	7·98	9·92	0·71
Average ..	200	7·38	10·04	0·78

El-Sokkary and Hassan (1950) report the following average values—

	Lactose	*Chloride*
Cow	4·71	0·0765
Buffalo	4·87	0·0649

The freezing point was found to be about 0·01° C below that of cows' milk.

The composition of the fat of buffaloes' milk has been examined by Hogan and Griffiths-Jones (1916 and 1919) who worked with 69 samples each from the mixed milk of five or six animals. The following results were obtained—

	Reichert-Meissl value	*Polenske value*	*Saponification value*	*Iodine value*	*Zeiss number* 40° C
Maximum ..	37·0	2·8	235	39·7	44·0
Minimum ..	24·5	1·0	218	23·0	40·4
Mean	31·2	1·5	229	31·4	42·5

Richmond held the opinion that the sugar is lactose and that the proteins are similar to those of cows' milk.

The fatty acids and component glycerides of the fat of the Indian buffalo have been examined by Bhattacharya and Hilditch (1931) who found distinctly more butyric acid than in cows' milk fat and the proportion of linoleic to oleic to be lower, whilst the amount of stearic acid was considerably higher, and small but definite amounts of arachidic acid were isolated (Pissarewsky 1936). (For ghee from buffalo and other milk fats, see p. 258.)

Other authorities have obtained data given in Table 4.9—

Table 4.9—Composition of fat of buffaloes' milk

Authority	Reichert value	Iodine absorption	Saponification value	Insoluble fatty acids	Soluble fatty acids	Polenske value
Strohmer ..	30·4	—	222·4	—	—	—
Pappel and Richmond—						
Winter ..	25·4	35·0	220·4	87·5	6·09	—
Summer ..	34·7	32·0	231·7	86·9	6·99	—
J. N. Dutt ..	34·5	—	—	—	—	—
Hogan and Griffiths-Jones	31·2	31·4	229	—	—	1·5
Bolton and Revis	30·8	30·3	228·9	—	—	—
Trimen ..	33·0	—	229·6	—	—	1·8

Goats' milk

The milk of goats is largely used in some parts of the world, notably in Gibraltar, where legal limits are in force of 3·5 per cent of fat and 8·0 per cent of solids-not-fat (Holborow 1928, 1929, 1931 and 1933). Goats' milk differs in certain respects from that of cows, notably in the behaviour of the proteins towards ammonia, in the potassium content of the ash, and in the composition of the fat. The composition of the milk has been variously given by several authors, as set out in the following table—

Table 4.10—Percentage composition of goats' milk

Authority	Total solids	Fat	Solids-not-fat	Sugar	Proteins	Ash	Remarks
H. D. Richmond	13·24	3·78	9·46	4·49	4·10	0·87	—
H. Hagar (1911)	12·8	3·8	9·0	—	—	—	av. of 200.
G. Hogan and A. Azadian (1919, 1922)	12·54	4·04	8·50	—	—	—	av. of 104; Egyptian.
A. G. Holborow (1928)	13·17	4·16	9·01	—	—	—	Gibraltar.
A. G. Holborow	14·35	4·32	10·03	—	—	—	av. 117 Gib.
A. G. Holborow	13·07	4·18	8·89	—	—	—	,, 47 ,,
A. Azadian ..	12·54	4·04	8·50	—	—	—	—
J. M. Clavera & D. Guevara	—	3·2 to 5·5	—	—	—	0·65 to 0·85	—
D. R. Dhingra (1933)	—	3·2 ,, 3·6	—	—	—	—	—

Buttenberg and Tetzner (1904) carried out analyses on the milk of five individual goats over seven consecutive days with the following results—

Table 4.11—Composition of goats' milk

Age of goat	Time	Fat per cent	Solids-not-fat per cent
2 years	M. E.	3·8 to 4·4 4·9 ,, 5·6	8·82 to 9·07 8·59 ,, 8·99
2½ years	M. E.	3·4 ,, 3·7 4·0 ,, 4·7	8·19 ,, 8·53 8·14 ,, 8·59
3 years	M. E.	2·5 ,, 3·4 3·0 ,, 3·9	8·01 ,, 8·43 8·18 ,, 8·52
3 years	M. E.	2·6 ,, 3·0 3·1 ,, 3·4	8·02 ,, 8·23 7·91 ,, 8·38
5 years	M. E.	2·1 ,, 2·5 2·3 ,, 2·8	7·13 ,, 7·71 7·37 ,, 7·59
AVERAGE	—	3·42	8·19

Hagar (1911) showed that the casein of goats' milk does not dissolve in ammonia, and Pritzker (1915) made use of this property for the detection of goats' milk in cows' milk. Nottbaum (1933) showed that goats' milk is particularly high in chlorine, 39 samples containing from 0·107 per cent to 0·185 per cent, with an average of 0·141 per cent. Schultz and Chandler (1921) found a pH of 6·7 to 6·4, average 6·53. They state that goats' milk rarely forms a cream layer on account of the very small size of the fat globules, of which 57 per cent have a diameter of less than 2 μ, 34 per cent from 2 to 4 μ, and 7 per cent 4 to 6 μ. Holborow (1928) points out that all goats' milk sold in Gibraltar must be boiled, and he deals with the detection of unboiled goats' milk in boiled goats' milk. Fonzes-Diacon (1922) stated that the simplified molecular constant for goats' milk is similar to that of cows' milk containing 10 per cent of water, but that the s.m.c. will distinguish the one from the other. A comprehensive review of the fat content of milk from British breeds of goats was undertaken by Urquhart (1930); the first two tables on page 99 are taken from Urquhart's paper.

It may be safely assumed that average goats' milk contains about 4 per cent of fat and somewhat over 9 per cent of solids-not-fat, i.e. that it is similar to, though slightly richer in both constituents than cows' milk. The small number of complete examinations that have been made suggests that the amounts of sugar, proteins and ash present are not very dissimilar from those contained in cows' milk.

Table 4.12—Goats' milk: average percentage of fat in morning and evening milks, 1919–1929

Breed	*Morning*	*Evening*
British	3·90	4·54
British Toggenburg	3·81	4·24
Toggenburg	3·24	3·75
British Saanen	4·00	4·34
Saanen	3·67	3·86
British-Alpine	4·03	4·35
Anglo-Nubian	4·98	5·66
All breeds	4·00	4·51

Table 4.13—Goats' milk: maxima and minima of fat in morning and evening milks, 1919–1929

Breed	Fat percentage			
	Morning		Evening	
	Max.	Min.	Max.	Min.
British	7·55	1·75	8·20	2·00
British Toggenburg	6·12	2·20	6·80	2·31
Toggenburg	5·18	2·05	6·25	2·50
British Saanen	6·95	1·90	7·30	1·90
Saanen	4·78	2·70	5·30	2·50
British-Alpine	7·10	1·60	7·10	2·60
Anglo-Nubian	8·85	2·30	9·09	3·60
All breeds	8·85	1·60	9·09	1·90

Lythgoe (1940a) gives the following more complete analyses for the individual milk of 21 goats—

Table 4.14—Percentage composition of goats' milk

	Highest	Lowest	Average
Total solids	18·40	11·96	14·42
Fat	7·25	2·95	4·93
Solids-not-fat	11·17	8·39	9·49
Lactose	5·65	4·25	4·78
Total proteins	5·62	3·38	4·11
Albumin	1·37	0·77	1·06
Ash	1·04	0·75	0·89
Freezing-point depression	0·595° C	0·550° C	0·573° C

Besley (1940) has surveyed goats' milk from the nutritional point of view and emphasised those points on which goats' milk differs from cows' milk. A detailed study of goats' milk has been published by Lythgoe (1940b) who reports the average freezing point to vary from —0·57 to —0·59° C. Phosphatase is also destroyed in 15 min. at 142° F (see p. 53).

The composition of the fat of goats' milk differs from that of cows' milk by having a very considerably higher Polenske value. The following results have been published—

Table 4.15—The fat of goats' milk

Authority	Refraction at 40° C			Saponification value			Reichert value			Polenske value		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
T. G. Stathopoulo	41·4	40·6	41·0	230·9	228·1	229·5	24·7	21·7	23·2	4·0	1·9	3·08
F. Knowles and J. C. Urquhart	45·0	42·4	43·9	—	—	—	27·8	24·5	26·5	8·7	4·9	7·3
R. C. Lopez	—	—	41·6	—	—	239·9	—	—	24·2	—	—	7·1
A. Azadian	41·7	41·2	—	244·0	212·0	—	24·4	22·4	—	4·6	3·3	—
J. König	—	—	—	—	—	—	29·0	17·0	—	9·8	3·2	—

Authority	Iodine value			Kirschner value		
	Max.	Min.	Av.	Max.	Min.	Av.
F. Knowles and J. C. Urquhart . .	37·0	24·7	31·2	19·0	16·8	17·5
A. Azadian	29·0	20·7	—	—	—	—
R. C. Lopez	—	—	26·2	—	—	—

The chief characteristic of the fat of goats' butter is the high Polenske value, which may rise, in certain cases, nearly as high as 11. An average figure is probably about 6. The Reichert value is somewhat lower than that of cows' butter fat, being about 25. Knowles and Urquhart have pointed out that the equations used for cows' butter fat are not applicable to the fat of goats' butter.

Bergman and Turner (1937) have dealt very fully with the colostrum of goats. They have reviewed a large proportion of the previous work and, as a result, give the following average figures for the colostrum of the dairy goat—

Table 4.16—Goats' colostrum (per cent)

Day	Total solids	Fat	Protein	Casein	Albumin	Globulin	Lactose	Ash
1	24·9	10·9	9·1	4·1	2·8	4·6	3·5	1·0
2	16·1	6·2	4·4	3·5	1·2	1·0	4·3	0·8
3	14·6	5·4	4·3	2·6	1·0	0·5	4·4	0·9
4	15·3	5·4	4·1	2·6	0·9	0·5	4·9	0·9
5	14·0	4·7	4·2	2·7	0·8	0·5	4·7	0·7
6	14·4	4·5	4·0	2·6	0·8	0·5	4·7	0·8
7	14·6	4·5	4·1	2·4	0·8	—	4·8	0·9

The colostrum of six goats was examined by Bergman and Turner, who found the following figures—

Table 4.17—Goats' colostrum (per cent)

Day	Total solids	Fat	Protein	Casein	Casein-globulin	Albumin	Globulin	Lactose	Ash
1	18·8	8·2	5·7	3·1	4·9	0·8	1·8	3·4	0·98
2	14·7	5·1	4·2	3·2	3·6	0·6	0·4	3·8	0·90
3	14·0	4·6	3·6	2·8	3·2	0·4	0·4	4·2	0·96
5	13·3	3·9	3·5	2·8	3·1	0·4	0·3	4·2	0·95
7	13·1	4·0	3·5	2·9	3·1	0·4	0·2	4·2	0·96
9	13·6	4·3	3·3	2·9	3·0	0·3	0·1	4·5	0·83

The component fatty acids of goats' milk fat have been examined by Reimenschneider and Ellis (1936) who found (per cent): butyric, 2·1; caproic, 1·9; caprylic, 2·7; capric, 7·9; lauric, 3·5; myristic, 10·2; palmitic, 28·7; stearic, 8·1; decenoic, 0·2; tetradecenoic, 0·4; hexadecenoic, 2·1, and oleic, 31·2. In addition, 0·4 per cent of arachidonic acid and a trace of an unknown acid, either a C_{22} acid or an isomer of arachidonic acid, were also found (Chollet and Camus 1938, Lythgoe 1939).

Camels' milk

The milk of the camel contains on the average 3·1 per cent of fat, 4·0 per cent of proteins, 5·6 per cent of sugar and 0·75 per cent of ash. The fat has been examined by Dhingra (1934) and Laxa (1934). The following figures were obtained—

Table 4.18—Fat of camels' milk

Determination	Dhingra	Laxa
Reichert value	16·4	1·5
Polenske value	1·6	0·9
Kirschner value	14·3	—
Iodine value	40·8	44·5
Saponification value	216·7	196·8

There is a very striking difference between these two results; it would appear that either one or the other is distinctly abnormal.

Ewes' milk

Ewes' milk has been examined by a number of workers. Besana (1893) found the fat to vary from 7·1 to 10·4 per cent, with an average of 8·92 per cent; proteins, 5·8 to 6·8 per cent, average 6·24 per cent; sugar, 4·9 to 5·2 per cent, average 5·05 per cent; ash, 0·89 to 1·09 per cent, average, 1·06 per cent. Trillat and Forestier (1902) quoted the mean results obtained by previous workers as: fat, 4·2 per cent; lactose, 4·0 per cent; casein, 3·7 per cent and ash, 0·7 per cent, but state that all these are too low. For 171 individual samples and for the averages from four different dairies they obtained the following results—

Table 4.19—Percentage composition of ewes' milk

Constituent	*Individual samples*	*Four dairies*
Fat	6·21 to 7·76	6·98 to 7·42
Sugar	4·83 ,, 5·45	5·26 ,, 5·53
Proteins	5·01 ,, 5·95	5·12 ,, 6·18
Ash	0·92 ,, 1·03	0·89 ,, 1·02

Pierce (1934) found that the average daily yield from 6 merino ewes was 1,205 g during the third week of lactation and 650 g during the ninth week. The average compositions at these two periods were—

Table 4.20—Percentage composition of ewes' milk

	Period of lactation	
Constituent	*3rd week*	*9th week*
Fat	7·41	7·90
Sugar	4·83	4·81
Proteins	4·24	5·23
Ash	0·86	0·90

Further results are given by other workers, these being summarised in a useful paper by Godden and Puddy (1935).

The fat of ewes' milk is similar to that of cows' milk. Stathopoulo (1933) examined seven authoritative samples of ewes' butter collected in the country districts of Greece and found: butyro-refractometer reading at 40°, 42·3 to 40·9, average 41·63; saponification value, 228·0 to 223·8, average 226·4; iodine value, 31·1 to 28·7, average 30·02; Reichert value, 23·4 to 22·8, average 23·0; Polenske value, 2·12 to 1·51, average 1·83.

Mares' milk

The early knowledge of mares' milk is due to Vieth (1883, 1884 and 1885), who carried out a series of observations on the stud mares at the International Health Exhibition in London during the year 1884. Vieth describes the milk as of a chalky-white colour, sweet and at the same time somewhat harsh in taste, and of aromatic flavour. Richmond points out that as the milk undergoes alcoholic fermentation very easily, while cows' milk does not, there is reason to suppose that the sugar is not identical with lactose. Vieth's results, with some others, are given in Table 4.21.

Linton (1931) examined the milk of 142 British mares and obtained the following results (per cent): total solids, 5·93 to 18·74, average 10·96; fat, 0·09 to 7·88, average 1·59; proteins, 0·55 to 7·60, average 2·69; lactose, 1·65 to 8·78, average 6·14; ash, 0·28 to 0·95, average 0·51. Hildebrandt (1918) carried out the examination of 21 samples taken during six weeks from the milk of three mares. The following results were obtained (per cent): total solids, 8·84 o 12·03; fat, 0·10 to 3·35; ash, 0·32 to 0·74; lactose, 4·32 to 7·56; nitrogen, ·31 to 0·49: proteins = 1·98 to 3·13. Barthe and Dufilho (1927) found a ontent of from 0·057 to 0·012 per cent of combined chlorine.

Table 4.21—Percentage composition of mares' milk (*Vieth and others*)

	Water	Fat	Sugar	Protein	Ash
Mixed milk					
Average	90·06	1·09	6·65	1·89	0·31
Maximum	90·41	1·44	6·82	2·11	0·34
Minimum	89·74	0·87	6·30	1·71	0·29
Milk of individual mares					
Average	90·13	0·94	6·98	1·65	0·30
Maximum	90·46	1·18	7·21	1·76	0·36
Minimum	89·88	0·62	6·70	1·51	0·26
Milk of mares specially fed					
Average	89·22	1·48	7·03	1·99	0·28
Maximum	89·88	2·14	7·28	2·20	0·32
Minimum	88·24	1·18	6·67	1·70	0·24
Fleischmann gives the following composition—					
Average	90·7	1·2	5·7	2·0	0·4
Maximum	92·53	2·45	7·26	3·00	1·20
Minimum	89·05	0·12	4·20	1·33	0·28
The following composition is also given—					
Authority					
Landowsky	89·29	1·16	7·32	1·87	0·36
Biel	90·42	1·31	5·43	2·55	0·29
Camerer and Söldner	90·58	1·14	5·87	2·05	0·36
Vieth gives the following composition of samples of condensed mares' milk (containing cane sugar 16 to 18 per cent)—					
(1)	26·73	4·77	53·07	13·69	1·74
(2)	24·04	6·20	55·81	12·17	1·78
(3)	17·90	12·07	54·88	13·50	1·65
(4)	18·80	10·08	54·09	15·23	1·80

Linton (1927) in a subsequent investigation has found that in this milk, as in others, the stage of lactation is the principal factor governing composition; with advancing lactation, total solids, lactose and fat increased, whilst the solids-not-fat and proteins decreased.

Laxa (1928) has examined the mixed fat of the milk from two mares and has found the saponification value to be 227·8; butyro-refractometer reading at 40°, 50·0; iodine value, 61·0; and Reichert value, 7·0.

Sows' milk

A useful survey of existing knowledge is given by Braude in Davis's *Dictionary of dairying*. O. Laxa (1931) found that the fat of sows' milk had: saponification value 193·9; iodine value 58·2; Reichert value, 2·1; Polenske value, 1·2.

The milk of other mammalia

The following table gives the composition of milks of various mammals—

Table 4.22—Percentage composition of milks of various mammals

Source	Fat	Sugar	Proteins	Ash
Bitch	9·57	3·09	11·15	0·73
Cat	3·33	4·91	9·08	0·58
Elephant	19·57	8·84	3·09	0·65
Llama	3·15	5·60	3·90	0·80
Porpoise	48·50	1·33	11·19	0·57
Rabbit	10·45	1·95	15·54	2·56
Sow	4·55	3·13	7·23	1·05
Vixen[*]	6·30	4·56	6·25	0·96
Vixen[†]	12·25	3·96	17·04	2·34
Whale	43·67	7·11		0·46
Yak[‡]	6·09	—	—	—

[*] E. G. Young and G. A. Grant (1932).
[†] O. Laxa (1930).
[‡] A. Z. Tierärzt (1931).

Recent analyses are summarised in Table 4.23, and the characteristics of the fats of the milks of the ewe, goat and mare in Table 4.24.

Table 4.23—Average analytical data for milks of various mammals

Source	Total solids	Fat	s.n.f.	Total nitrogen	Lactose	Ash	pH	Density	Protein	Sp. gr.	
Ass		2·43	8·44	0·315	6·07	0·41	7·28	1·0318			Gonzales-Diaz and Gravioto (1948)
Ass		0·54–0·71	7·14–8·5	0·25–0·28	5·85–6·5	0·37–0·47					Anantakrishnan (1941)
Buffalo (Egyptian)		7·11	9·63								Ghoniem *et al.* (1948)
Buffalo (Indian) ..		2–12	8·5–15·5		4–5·3			1·028–1·040			Ghosh and Datta Roy (1942)
Monkey		3·9			5·9	0·26		1·017	2·1		van Wagenen *et al.* (1942)
Dog	22·6	8·3			3·7	1·2			7·5		Andersen *et al.* (1940)
Dolphin		10·8–18		1·25–1·9	0·4–0·77		6·6–7·1		9·4–11·1	1·01–1·02	Eichelberger *et al.* (1940)
Elephant	26·85	15·1			3·4	0·76			4·9	1·0315	Nottbohm (1939)
Goat	11·71	3·5			4·55	0·79			3·10		Gamble *et al.* (1939) comprehensive paper
,, ...	14·1	4·9	9·2		4·17	0·98			3·99		Bagnall (1943)
,, ...		4·7–6·6	8·3–9·2								Whittle (1943)
,, ...		3·91	9·5								Trout (1941a)
Mare (winter) ..	11·23	1·32			6	0·48			2·10		Papp (1941)
Mare (summer) ..	10·36	0·86			5	0·31			1·98		

Table 4.24—Analysis of fat in milk of mammals

Source	Refractometer number	Saponification value	Iodine value	Reichert–Meissl	Polenske	Kirschner	Solidifying point ° C	Melting point ° C
Ewe (*a*) . .		244·5	36·7	26·2	3·6	17·6		
Goat (*a*) . .		233·7	28·8	23·2	7·2	15·6		
(*b*) . .	42·5	240·6	29·6	26·8	4·8		17·6	35·5
Mare (*a*) . .		253·1	84·3	6·2	5·9	2·6		

(*a*) Hilditch and Jasperson (1944).
(*b*) Izmen (1949).

CHAPTER 5

THE PHYSICAL EXAMINATION OF MILK

(1) SPECIFIC GRAVITY AND DENSITY

The density of a substance may be defined as its mass per unit volume at a specified temperature. The specific gravity of a substance is the ratio of its mass at a known temperature to the mass of an equal volume of water at 4° C or other specified temperature. The unit of volume normally used is the millilitre and the unit of weight the gram. As 1 millilitre of water at 4° C (3·98° C) weighs 1 gram, the specific gravity of water at 4° C is exactly 1; at temperatures higher and lower than 4° C, water expands and therefore has a specific gravity, relative to 4° C, of less than 1. Table 5.1 gives the specific gravity of water at different temperatures relative to water at 4° C—

Table 5.1—Specific gravity of water

Temperature °C	Specific gravity	Temperature °C	Specific gravity
0	0·99988	40	0·99236
4	1·00000	50	0·98817
10	0·99974	60	0·98334
15·5 (60° F)	0·99908	70	0·97789
20	0·99827	80	0·97190
30	0·99577	90	0·96549
37·8 (100° F)	0·99313	100	0·95856

The above figures are compared with water at its point of maximum density, 4° C; in practice, however, it is customary, from the convenience of carrying out determinations of specific gravity at average room temperature, to assume that water at 15·5° C (60° F) has a specific gravity of 1.

Thus, to ascertain the specific gravity at 15·5° C it is customary to weigh a known volume of liquid, and to compare it with the weight of an equal volume of water at the same temperature, both in air. All specific gravities in the present work are stated in this way unless otherwise mentioned. In order to avoid confusion, the term " specific gravity at 15·5°/15·5° " is often used to denote this mode of expression. This means that the weight of a volume of liquid at 15·5° C is compared with the weight of an equal volume of water at 15·5° C. Similarly, the expression " specific gravity at 15·5°/4° or 20°/4° " is used to denote the true specific gravities at 15·5° or 20°.

Occasionally it is convenient to compare the weight of a liquid at some other temperature with the weight of water at that temperature; thus the specific gravity of fats may be taken at 100° C, and the expression "specific gravity at 100°/100°" is used to express the value obtained by dividing the weight of a volume of fat at 100° C by the weight of an equal volume of water at 100° C.

If we ascertain the weight of water held by a certain vessel at a definite temperature, we can ascertain the specific gravity of any liquid by filling it with the liquid at the same temperature and weighing it. If we fill the vessel with the liquid at any other temperature, the volume contained will not be the same as that of the water, owing to the expansion of the vessel itself altering the capacity. Nevertheless, specific gravity is frequently ascertained on the assumption that the vessel does not alter in capacity by change of temperature. As the vessel is usually made of glass, this mode of expression may be termed the "apparent specific gravity in glass at 20°/15·5°" (or whatever the temperature may be).

As a matter of fact, specific gravities of milk are usually determined as "apparent specific gravities in glass at $\frac{x° \text{ C}}{15{\cdot}5° \text{ C}}$ or $\left(\frac{x° \text{ F}}{60° \text{ F}}\right)$."

Variation of specific gravity of milk

The specific gravity at 15·5° C of the milk of individual cows varies from 1·0135 to 1·0397; when the mixed milk of a herd is tested it rarely falls outside the limits of 1·030 and 1·034. The average value is 1·0320.

The specific gravity is dependent on two factors: the amount of solids-not-fat which, being dissolved in water, raise the specific gravity; and the fat which, being lighter than water, lowers it. By removing the fat (with a small proportion of other constituents) as cream, the specific gravity is raised. By the addition of water the specific gravity is lowered. The specific gravity has been—and is—largely used as a test for the addition of water to milk, and for the detection of large amounts of added water it has some value. That this is, however, a test of the roughest kind is shown by the following facts—

(1) The variations in specific gravity are from 1·0135 to 1·0397, i.e. nearly twice its bulk of water could be added to milk of the highest specific gravity to reduce it to the lowest. These, of course, are exceptional cases, and the specific gravity of the mixed milk of a herd is nearly always between 1·030 and 1·034. At least 10 per cent of water could be added to milk of 1·034 specific gravity before it would be suspected by this test.

(2) A milk of 1·032 specific gravity, if the cream is all removed, would give a product of about 1·036 specific gravity; and an addition of rather more than 10 per cent of water would bring the specific gravity back to 1·032.

(3) If to milk of 1·032 specific gravity sufficient cream be added to raise the percentage of fat 4 per cent the specific gravity will be found to be about 1·028.

As an absolute test, specific gravity is liable to be misleading, but as a preliminary test it should never be neglected.

Specific volume

This is also used in the determination of the average specific gravity of milk samples.

Specific volume is the inverse of specific gravity: if S = specific gravity, then $\frac{1}{S}$ = specific volume; and it expresses the volume of unit mass of the substance. Degrees of specific gravity on lactometer scales are represented as 1,000 times the specific gravity minus 1,000; degrees of specific volume, on the other hand, are represented by 1,000 minus 1,000 times the specific volume.

In Table 5.2 the values of degrees of specific volume for each half-degree of specific gravity from 20 to 36 are given.

Table 5.2—Specific gravity and volume of milk

Degrees of specific gravity	Degrees of specific volume	Degrees of specific gravity	Degrees of specific volume
20·0	19·6	28·5	27·7
20·5	20·1	29·0	28·2
21·0	20·6	29·5	28·7
21·5	21·0	30·0	29·1
22·0	21·5	30·5	29·6
22·5	22·0	31·0	30·1
23·0	22·5	31·5	30·5
23·5	23·0	32·0	31·0
24·0	23·4	32·5	31·5
24·5	23·9	33·0	31·9
25·0	24·4	33·5	32·4
25·5	24·9	34·0	32·9
26·0	25·4	34·5	33·4
26·5	25·8	35·0	33·8
27·0	26·3	35·5	34·3
27·5	26·8	36·0	34·7
28·0	27·2		

It can be shown that 1 per cent by weight lowers the specific volume to the same extent as 1 g per 100 ml raises the specific gravity; i.e. specific volume, not specific gravity, varies directly as percentage by weight. It follows, therefore, that it is not correct, in averaging milk analyses where specific gravities and percentages by weight are expressed, to obtain the average specific gravity by simply adding the specific gravities together and dividing by the total number. The specific gravities must first be calculated to specific volumes, and these averaged, and the average specific gravity deduced from the average specific volume.

Thus, to average the following analyses—

Specific gravity	... 1·022	Total solids	... 20·0
,, ,,	... 1·036	,, ,,	... 10·0

the average total solids is $\frac{20 + 10}{2} = 15$; but the average specific gravity is not

$\frac{1\cdot022 + 1\cdot036}{2} = 1\cdot029$, but $\frac{1}{\frac{0\cdot9785 + 0\cdot9653}{2}} = \frac{1}{0\cdot9719} = 1\cdot0289$. The error is, however, small if the specific gravities do not differ greatly, and may usually be neglected.

On the other hand, if, instead of averaging percentages of total solids by weight, we average the number of grams per 100 ml we obtain correct results by averaging the specific gravities.

The following rules may be stated—

(1) If equal volumes of different milks be mixed, the specific gravity of the mixture will be the mean of the specific gravities of the milks.

(2) If equal weights of different milks be mixed, the specific volume of the mixture will be the mean of the specific volumes of the milks.

The alteration of specific gravity by change in temperature

Milk, like all other substances, alters in specific gravity by change of temperature, but, though it contains a large amount of water, it does not share the anomaly which water possesses of attaining its maximum specific gravity at 4° C (39° F). It decreases in specific gravity when heated from its freezing point, –0·55° C (31° F).

The following figures show the average apparent expansion of milk in glass—

Table 5.3—Expansion of milk

Temperature in °F	Volume	Temperature in °F	Volume
31	1·00000	60	1·00229
35	1·00016	65	1·00298
40	1·00041	70	1·00372
45	1·00074	75	1·00451
50	1·00114	80	1·00549
55	1·00164		

The expansion is greater with rich milk than with poor milk, the above figures referring to milk having a specific gravity of 1·032 and containing 3·8 per cent of fat.

Table 5.4 affords a means of correcting to 60° F the specific gravity of milk when taken by a lactometer at any temperature from 40° F to 80° F. The table gives specific gravities from 1·025 (25 degrees) to 1·036 (36 degrees) and is applicable to whole milk only.

The table is used by looking up the degrees of specific gravity found (or the nearest whole degree) in a horizontal line, and the temperature in a vertical line; the figure at the intersection of the two lines is the correction to be added if above 60°, and subtracted if below, to reduce the specific gravity to 60° F.

The specific gravity of separated milk may be corrected to 60° F by using the column for 31° (between the black lines); the reason for using this, instead of the column proper to the specific gravity, is that the separated milk, being free from fat, has a smaller expansion than normal milk.

Table 5.4—For correcting the specific gravity of milk to 60° F

Temperature Degrees F		Degrees of specific gravity observed											
		25	26	27	28	29	30	Skim 31	32	33	34	35	36
		Corrections to reduce degrees of specific gravity to 60° F											
40	Subtract from reading	1·5	1·5	1·5	1·6	1·7	1·7	1·9	2·0	2·0	2·1	2·2	2·3
41		1·4	1·4	1·4	1·5	1·6	1·7	1·8	1·9	2·0	2·0	2·1	2·2
42		1·4	1·4	1·4	1·5	1·5	1·6	1·7	1·8	1·9	2·0	2·1	2·1
43		1·3	1·3	1·3	1·4	1·4	1·5	1·6	1·7	1·8	1·9	2·0	2·0
44		1·2	1·2	1·3	1·3	1·3	1·4	1·5	1·6	1·7	1·8	1·9	1·9
45		1·2	1·2	1·2	1·3	1·3	1·4	1·5	1·6	1·6	1·7	1·8	1·8
46		1·1	1·1	1·2	1·2	1·2	1·3	1·4	1·5	1·6	1·7	1·7	1·7
47		1·0	1·1	1·1	1·2	1·2	1·3	1·4	1·5	1·5	1·5	1·6	1·6
48		1·0	1·0	1·0	1·1	1·1	1·2	1·3	1·4	1·4	1·4	1·5	1·5
49		0·9	0·9	0·9	1·0	1·0	1·1	1·2	1·3	1·3	1·3	1·4	1·4
50		0·9	0·9	0·9	1·0	1·0	1·0	1·1	1·1	1·2	1·2	1·3	1·3
51		0·8	0·8	0·8	0·9	0·9	0·9	1·0	1·0	1·1	1·1	1·2	1·2
52		0·7	0·8	0·8	0·8	0·8	0·9	0·9	0·9	1·0	1·0	1·1	1·1
53		0·6	0·7	0·7	0·7	0·7	0·8	0·8	0·8	0·9	0·9	1·0	1·0
54		0·5	0·6	0·6	0·6	0·6	0·7	0·7	0·7	0·7	0·7	0·8	0·9
55		0·4	0·5	0·5	0·5	0·6	0·6	0·6	0·6	0·6	0·6	0·7	0·7
56		0·4	0·4	0·4	0·4	0·4	0·4	0·5	0·5	0·5	0·5	0·6	0·6
57		0·3	0·3	0·3	0·3	0·3	0·3	0·4	0·4	0·4	0·4	0·5	0·5
58		0·2	0·2	0·2	0·2	0·2	0·2	0·2	0·3	0·3	0·3	0·3	0·3
59		0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·2	0·2
60		**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
61	Add to reading	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1
62		0·2	0·2	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3
63		0·3	0·3	0·4	0·4	0·4	0·4	0·4	0·5	0·5	0·5	0·5	0·5
64		0·4	0·5	0·5	0·5	0·5	0·5	0·5	0·6	0·6	0·6	0·6	0·6
65		0·5	0·6	0·6	0·6	0·6	0·7	0·7	0·7	0·8	0·8	0·8	0·8
66		0·6	0·7	0·7	0·7	0·8	0·8	0·8	0·9	0·9	0·9	0·9	1·0
67		0·7	0·8	0·8	0·8	0·9	0·9	1·0	1·0	1·0	1·0	1·1	1·1
68		0·9	1·0	1·0	1·0	1·1	1·1	1·1	1·2	1·2	1·2	1·2	1·2
69		1·0	1·1	1·1	1·1	1·2	1·2	1·2	1·3	1·3	1·3	1·4	1·4
70		1·1	1·2	1·2	1·2	1·3	1·3	1·4	1·4	1·5	1·5	1·5	1·6
71		1·2	1·3	1·3	1·4	1·4	1·5	1·5	1·6	1·6	1·6	1·7	—
72		1·4	1·4	1·4	1·5	1·5	1·6	1·6	1·7	1·7	1·8	1·8	—
73		1·5	1·5	1·6	1·7	1·7	1·8	1·8	1·9	1·9	2·0	2·0	—
74		1·6	1·7	1·7	1·8	1·9	1·9	2·0	2·1	2·1	2·2	2·2	—
75		1·8	1·8	1·9	1·9	2·0	2·1	2·2	2·3	2·3	2·4	2·4	—
76		1·9	1·9	2·0	2·0	2·1	2·2	2·3	2·4	2·4	2·5	2·6	—
77		2·0	2·0	2·1	2·2	2·3	2·4	2·4	2·5	2·6	2·7	—	—
78		2·2	2·2	2·2	2·3	2·4	2·5	2·6	2·7	2·8	2·9	—	—
79		2·3	2·3	2·4	2·4	2·5	2·6	2·7	2·8	3·0	3·0	—	—
80		2·4	2·4	2·5	2·6	2·7	2·8	2·9	3·0	3·1	3·2	—	—
		25	**26**	**27**	**28**	**29**	**30**	Skim **31**	**32**	**33**	**34**	**35**	**36**

Richmond has devised a scale for correcting the specific gravity of milk to 60° F. It is usually engraved on the " milk scale ", and is used by adjusting the specific gravity found (on the slide) to the arrow at 60° F. The corrected specific gravity is found opposite the temperature at which the determination was made. In practice it will be found more convenient in the laboratory to use the " milk scale " (see p. 160) than to refer to tables.

The corrected specific gravities obtained by the " milk scale " agree generally within 0·1 of those taken from the table. At very low temperatures, however, there is sometimes a larger difference.

Collins has devised a milk scale in which the temperature-correction for specific gravity is made automatically; the points denoting specific gravity and temperature observed are brought together, and on the other side of the scale the percentage of solids-not-fat corresponding to any percentage of fat is read off.

The rise of specific gravity of milk on standing

Milk drawn from the udder contains a large number of air bubbles, and its specific gravity cannot be taken; after the expiration of an hour or so these have disappeared and a specific gravity determination is possible. It was first observed by Recknagel that the specific gravity taken after the expiration of one hour was lower than the specific gravity subsequently obtained. He found the rise in specific gravity to be regular, more rapid at low temperatures than high ones, and to amount on the average to 0·001. He attributed the change to an alteration in the volume of the casein. Vieth confirmed Recknagel's observation completely, and found the average rise to be 0·0013; Bourcart also observed the phenomenon.

Richmond investigated " Recknagel's phenomenon " (as this change in specific gravity has been called); in about 70 per cent of his experiments the rise in specific gravity was observed, varying from 0·0015 to 0·0003, and averaging 0·0006, while in 30 per cent of the observations no rise in specific gravity was indicated. The experience of Babcock and Farrington agreed with that of Richmond. The latter's experiments confirmed the statement of Recknagel that the rise is more rapid when the temperature is low than when high; the same ultimate specific gravity is attained whatever the temperature.

The Recknagel change appears to be unconnected with the milk-sugar; and Recknagel's own explanation is not the correct one. It is also difficult to reconcile the idea that the change is enzymic with the fact that it is more rapid at low than at high temperatures. Richmond's experiments on the change of density and specific heat of cream by heating, made in conjunction with S. O. Richmond, showed that the effect is due largely to the increase of density of the fat on solidification.

Contrary to Richmond's former conjecture, there seems to be no particular period of the year in which Recknagel's phenomenon is observed. Samples have been found at all seasons which show a marked change in specific gravity, while others examined almost simultaneously have shown no change.

According to Boden and Campbell (1942) the Recknagel effect corresponds to a difference of 0·00075 in sp. gr., or about 0·8 " degrees of gravity ". The lower sp. gr. value for the uncooled milk agrees closely with the density obtained by B.S. No. 734—1937, both giving s.n.f. a value about 0·2 lower than the Richmond (aged and cooled milk) method, which agrees well with the gravimetric (evaporation) method. These authors point out that whereas the Richmond technique selected 10 per cent of a certain number of raw milk samples as being " less than 8·5 per cent ", the B.S. method returned about 25 per cent as being below the legal limit. The sp. gr. method tended to give higher values in November–July and lower in August–October. Their relation to the gravimetric results depended upon the particular evaporation method used.

Walker (1945) has also emphasised the importance of this ageing effect on the sp. gr. of milk, and has drawn attention to the fact that homogenising, with or without sterilising, may delay the rate of the Recknagel change, three days or more being necessary according to his figures. It should be clearly understood that the B.S. hydrometer and the Richmond lactometer give similar results by their respective formulae *if the milk is tested under the same conditions*. The Richmond method is usually used on aged and cooled milks, and the B.S. method on recently warmed milks, and for this reason the latter gives a lower value. While the B.S. method gives consistent results, the warming of milk samples in summer may lead to " buttering " or curdling, and the lower results obtained may lead to the conclusion that there has been a fall in s.n.f. in recent years.

It must be mentioned that Recknagel's phenomenon has been denied by some. Smetham attributes the change in specific gravity solely to the presence of air bubbles. The weight of evidence is, however, greatly against this view; it is inconceivable that air bubbles generated by milking a cow should be persistent for twelve hours, while if they are formed in the milk by other means, say by running through a separator, they disappear in one hour.

In practice milk inevitably becomes aerated in handling, pouring, pumping, etc., and this aeration in effect contributes to the Recknagel phenomenon. In tanker milk the *total* effect may be equivalent to as much as 0·15 per cent s.n.f.

The final specific gravity is always taken as the true specific gravity of milk, and the term is so used in this volume.

(2) REFRACTOMETRIC EXAMINATION OF MILK

The refractive index—or other similar figure on some arbitrary scale such as that of the immersion refractometer—has been used to some considerable extent, particularly in the United States of America, for the purpose of detecting added water in milk. Some workers have made exaggerated claims for this method, even stating that it is the most certain of the many which have been investigated. A little thought, however, will show that it has little if any advantage over the determination of any one of several constituents, which are only moderately constant between different milks and which are entirely useless for the purpose of detecting extraneous water in abnormal samples. Thus the immersion refractometric figures for genuine milks vary from about 36·5 to 40·0 at 20° and, obviously, a very considerable amount of water could be added to a genuine milk of high refractivity before the addition of water could be seriously suspected. Moreover it has been shown that the refraction figure is almost a linear function of the solids-not-fat, so that, in general, the indications given by these two methods will be similar in type and in amount.

According to Rangappa (1948) albumin and globulin affect the refractive index (n) of milk more than does casein. The relation of n and lactose concentration is not linear. Skimming both lowers and narrows the range of n for cow and buffalo milks, so that it is not possible to differentiate the two by this means (1949). Rangappa (1947) has also studied the contribution of the components of milk to its refractive index, and finds the order to be water > proteins > lactose > minor constituents. Albumin and globulin are more important than the casein. Values of $n = 1{\cdot}3474$ to $1{\cdot}3506$ were obtained for cows' milk and of 1·3484 to 1·3534 for buffalo milk.

Preparation of milk serum

On account of its physical condition, it is difficult to obtain consistent readings for the direct refraction of milk, particularly by the immersion refractometer—the most convenient, and therefore the most widely used instrument. It is necessary, therefore, to produce a milk serum. Various suggestions have been made for this purpose, among the more common of which are (1) spontaneous souring, or coagulating with either (2) acetic acid, (3) calcium chloride solution, (4) carbon tetrachloride and acetic acid, (5) copper sulphate solution, (6) phosphotungstic acid, or (7) dialysed iron. Clear sera have also been produced by filtration through " candles ". The perfect method of serum production has not yet been found. Except in the method of the filter candle and that of spontaneous souring, all the methods so far proposed entail the addition of a foreign material, the presence of which affects the figure obtained. The filter candle method suffers from the fatal objection that candles of different porosity give different results when used for the same milk. The relative advantages of some of the other methods of serum preparation will now be discussed.

(1) *Spontaneous souring*—The great advantage of this method is, of course, that no foreign substance is added to the milk; the disadvantages are that the milk must be received when fresh, that it must be kept under observation from time to time until curdling takes place, and that the process of souring cannot be controlled. Markedly different results may be obtained from the same milk allowed to sour at different temperatures, as is shown in Table 5.5.

The figures given in this section all refer to the immersion refractometer using prism I.

Table 5.5—Refraction of milk standing at different temperatures until sour

At 21° C No. of days	Refraction	At 17° C No. of days	Refraction	At 14° C No. of days	Refraction	At 7° C No. of days	Refraction
3	40·8	4	40·8	7	40·2	17	41·0
3	42·2	4	42·2	7	42·2	17	42·8
3	41·5	4	41·6	7	43·8	19	43·4
2	41·7	3	41·7	7	40·8	15	42·0
3	41·6	4	41·6	7	42·6	15	42·3
2	42·4	3	42·4	3	44·6	17	42·5

Elsdon and Stubbs (1930b) examined 351 samples of milk as soon as good coagulation had taken place. The preliminary thickening was ignored, as it was not found possible to obtain a clear filtrate until coagulation was fairly complete. The range of refraction found was from 39·6 to 44·1, with an average of 41·9. 344 of these samples had a range of 40·6 to 43·3, with the same average. The American Association of Official Agricultural Chemists' limiting figure for genuine milk is 38·3 at 20°.

(2) *Acetic acid.* This does not always produce a clot from which the serum can be easily filtered. It has no specific advantages. It is an official method of the American A.O.A.C., which gives the limiting figure for genuine milk as 40 at 20° C.

(3) *Calcium chloride solution.* This method has been extensively used on the continent of Europe, but appears to offer little if any advantage. The great disadvantage is that heating is necessary.

(4) *Carbon tetrachloride and acetic acid.* This method has been used on the continent of Europe. It appears to offer no particular advantage.

(5) *Copper sulphate solution.* This method is the one which has been used most extensively in England and the United States. It is easy to control and rapidly gives a clear serum. It suffers from the disadvantage that the milk must be quite fresh, otherwise high results will be obtained—caused by the fact that a portion of the proteins are already precipitated, so that less copper is removed from solution during the precipitation. The following figures indicate the differences which may be expected from this source—

Table 5.6—Refraction of milk (copper sulphate) on souring

Sample No.	Period in days				
	0	1	2	3	4
1	38·3	38·8	39·9	39·6	39·3
2	38·2	38·4	39·7	39·7	39·6
3	37·6	37·9	38·8	38·8	38·8
4	38·9	39·2	40·5	40·3	40·0
5	37·7	38·0	39·0	39·1	39·0
6	38·4	38·6	39·6	39·3	39·2

The drop, after the first rise, continued and is, of course, due to the decomposition of the lactose.

Any result below 37·0 raises the suspicion that extraneous water may be present in the sample.

(6) *Phosphotungstic acid.* This reagent gives a clear and bright serum. It does not suffer from the disadvantage of the copper serum of giving higher results when the milk is slightly sour. The range for genuine milks is usually between 33·4 and 36·1 at 20° with an average of 34·9. Figures as low as 32 have been obtained. As the whole of the albumen is removed, this method gives a lower result than that given by the copper sulphate method—the average difference being 3·2.

(7) *Dialysed iron.* A perfectly clear and bright serum can be obtained by mixing equal quantities of milk and dialysed iron. The disadvantage of the process is that it entails a considerable dilution of the milk, but it has the great advantage that the whole of the precipitant is removed along with the precipitate, leaving no foreign substance in the serum. No increase in the refraction is noticed when the milk becomes slightly sour. The range is about 25 to 27 with an average of 26·1. By calculation it can be shown that this average is very near, arithmetically, to that obtained with other precipitants, after allowing for extra dilution.

The figures likely to be obtained by the use of the most useful precipitants on separate portions of the same milk are given in Table 5.7—

Table 5.7—Refractions of milk serum prepared in different ways

Sample No.	Dialysed iron	Phospho-tungstic acid	Copper sulphate	Sour serum
1	26·1	34·7	38·3	41·6
2	26·2	34·8	38·2	42·0
3	24·5	32·0	35·5	37·9
4	25·9	34·7	37·9	41·8
5	26·0	34·6	38·1	41·7
6	26·2	35·2	38·4	42·2
7	26·1	35·2	38·6	42·9
8	26·0	35·1	38·4	41·8
9	26·0	34·9	38·3	42·0

The refraction of distilled water at 20° C—the temperature now used for such determinations—is 14·5. The difference between this figure and the refraction of a milk serum is the effect on the refraction caused by the soluble constituents in the milk serum. Apart from the effect due to the coagulant, which will vary according to the method used for preparing the serum, this difference figure is mostly due to the presence of lactose and alkali salts. The refraction of the coagulating solution is usually definitely fixed at the lowest figure likely to be given by genuine milk—for copper sulphate solution this is 36·0. When dialysed iron is used, this result cannot be achieved, since the whole of the coagulant is removed from the solution; the serum produced has therefore a much lower refraction, as is shown by the results given in Table 5.7.

Although it cannot be regarded as entirely free from objections, on account of the fact that accurate results cannot be obtained with sour milks, the use of copper sulphate solution as a coagulant can be recommended for general work. It has been used extensively in the United States and in this country; it is very convenient in use and open to no more criticism than any of the alternatives that have been suggested. Figures in this section refer to copper sulphate serum, unless otherwise indicated.

The refraction of genuine milk serum

The American A.O.A.C. takes the limit for the refraction of genuine milk serum as 36·0. Tocher examined 676 samples of the genuine milk of individual cows and found an average figure of 38·28. Of these samples, 10 gave a reading of less than 36·1, whilst 37 gave readings of less than 36·6. Elsdon and Stubbs have examined 997 samples of mixed milk by this method, obtaining the results set out in Table 5.8 below.

Table 5.8—Comparison of refraction and solids-not-fat

Solids-not-fat	Number of samples	Range	Average
8·4	5	36·6 to 37·1	36·78
8·5	16	37·0 ,, 38·3	37·68
8·6	27	37·1 ,, 39·0	37·76
8·7	47	37·2 ,, 38·5	37·92
8·8	107	37·2 ,, 39·2	38·03
8·9	138	37·0 ,, 39·3	38·20
9·0	189	37·6 ,, 39·0	38·35
9·1	172	37·3 ,, 39·4	38·42
9·2	128	37·3 ,, 39·2	38·60
9·3	86	37·6 ,, 39·4	38·73
9·4	53	38·2 ,, 39·4	38·87
9·5 and over	29	38·4 ,, 40·2	39·17
	997	36·6 ,, 40·2	38·35

As previously stated, the refraction varies to a considerable extent with the acidity. The figures given in Table 5.8 have therefore been rearranged under acidities instead of under the percentage of solids-not-fat, and the results are given in Table 5.9.

Table 5.9—Comparison of refraction and acidity

Acidity	Number of samples	Range	Average
1·5	17	37·1 to 38·8	37·89
1·6	24	36·6 ,, 38·8	37·83
1·7	69	37·1 ,, 39·0	38·00
1·8	159	37·0 ,, 39·2	38·25
1·9	186	37·0 ,, 39·3	38·32
2·0	197	37·3 ,, 39·3	38·39
2·1	137	37·0 ,, 39·4	38·51
2·2	96	37·1 ,, 40·2	38·67
2·3	36	38·1 ,, 39·4	38·74
2·4	28	37·4 ,, 39·4	38·71
2·5 and over	48	37·7 ,, 39·9	38·66
	997	36·6 ,, 40·2	38·35

From this table it appears that the refraction of a milk serum is almost as much a function of the acidity of the milk as it is of the solids-not-fat. With a fresh milk, however, it is known that high titratable acidity usually accompanies a high solids-not-fat percentage—this fact is shown in Table 5.10—

Table 5.10—Acidity and solids-not-fat

Acidity	Number of samples	Average solids-not-fat per cent
1·7	112	8·91
1·8	160	8·97
1·9	188	9·01
2·0	198	8·99
2·1	140	9·10
2·2	96	9·17
2·3 and 2·4	64	9·19

As acidity and solids-not-fat are thus related, the regularities shown in Table 5.9 are due to some extent to the same cause. For this reason, solids-not-fat have been correlated (Table 5.11) with refraction for each 0·1 increase in the acidity.

Table 5.11—Variations of refraction with solids-not-fat and acidity

Acidity	Solids-not-fat, per cent										
	8·5	8·6	8·7	8·8	8·9	9·0	9·1	9·2	9·3	9·4	9·5
1·7	—	37·76	37·75	37·74	38·08	38·26	38·29	38·21	38·55	—	—
1·8	37·57	37·58	37·92	38·03	38·26	38·24	38·44	38·53	38·70	38·83	38·83
1·9	—	37·88	38·14	37·95	38·19	38·39	38·37	38·50	38·56	38·69	—
2·0	—	—	38·14	38·22	38·20	38·35	38·38	38·53	38·61	38·77	39·00
2·1	—	—	38·25	38·13	38·00	38·47	38·39	38·69	38·71	38·81	38·95
2·2	—	—	—	—	38·21	38·50	38·44	38·60	38·62	38·96	39·23
2·3 and 2·4	—	—	—	—	38·46	38·61	38·59	38·79	38·89	38·91	39·13
2·5 to 3·0	—	—	—	—	38·20	—	—	38·88	38·56	—	39·27
Above 3·0	—	—	—	—	—	—	39·08	—	—	—	—

From these tables and Tocher's results it appears very improbable that the genuine milk of even small herds of cows will have a refraction of less than 37. The range for normal samples is 37·0 to 39·0. Abnormal samples may give results lower or even a little higher, and abnormal samples from individual cows may give results varying somewhat widely from these. A mixed milk giving a copper-sulphate serum refraction of less than 36·0 is probably watered, but a higher figure than this is no proof that a milk is genuine. In general, however, it can be stated that the method is of little if any greater value than the determination of the solids-not-fat, and it is certainly not possible to pass any definite opinion as to the authenticity of a sample of milk—whether from a herd or from an individual cow—on the indications of the refractometer alone.

The determination of refraction

The instrument to be preferred is the immersion refractometer of the pattern originally introduced by Zeiss. The readings are on an arbitrary scale − 5 to + 105), fractions of a division being ascertained by means of a micrometer adjustment. Using prism I, the one suitable for this purpose, the corresponding indices of refraction are 1·32539 to 1·36640. The instrument is supported on a stand, with the prism at the lower end, dipping into a small beaker containing the solution to be tested. The beaker is placed in a water-bath maintained at a constant temperature, usually 20° C, and so arranged that white light is reflected through the bottom of the beaker into the instrument. The regulation of the temperature is obtained by means of a constant flow of water through a spiral heater; the flow of gas should be controlled by means of a gas regulator, otherwise it may be difficult to obtain the necessary constancy to 0·1° C.

Copper sulphate serum

The copper sulphate solution is prepared by dissolving 71·5 g of A.R. copper sulphate in water and diluting to 1,000 ml. The resulting solution should have a refraction of exactly 36·00 at 20° C and must be adjusted to this, if necessary, by the addition of water or copper sulphate. For the coagulation, 20 ml of milk are mixed with 5 ml of the copper sulphate solution in a 6 × 1 in. test-tube. The mixture is well shaken and filtered through paper. Where more serum is required, larger quantities of milk and copper sulphate solution may be used, provided that the respective volumes are kept in this proportion. The result obtained is the same, whether the copper sulphate solution be added to the milk or the milk be added to the copper sulphate solution.

When a quantity of about 10 ml of serum has been obtained, it is placed in one of the small beakers, which is then placed in the temperature-controlled water-bath. When the serum has attained the temperature of 20° ± 0·1° C, the refraction is observed. For this purpose, a quantity as small as 1 ml may be used. The actual reading is not affected by the volume of liquid, but the line of demarcation is sharper when a larger volume is used. The reading should always be taken with the eye in the centre of the eyepiece. Even small quantities of liquid—two or three drops—may be examined by means of the closed beaker with the auxiliary prism which is supplied with the instrument. The results obtained agree with those obtained with the open beaker. In order that the temperature equilibrium be not disturbed by the putting of the prism into the serum, the refractometer should be kept in a beaker of distilled water, also maintained at 20° C. Immediately before use, the prism is removed from the water, dried with a clean dry duster, and then placed in the serum. A short time should be allowed to elapse before the reading is taken, and it is repeated after a further interval of 15 to 30 seconds; constancy of reading, with sharpness of definition, indicates that the prism has reached the temperature of the serum, which should be 20° C. Each time the refractometer is used, the reading should be checked by taking the refraction of distilled water, which is 14·5 at 20° C—different observers should make their own adjustments. Under such circumstances, consecutive readings can be made to within 0·05 of a division. The temperature should be kept as nearly as possible at 20° C. The refraction of milk serum is *increased* by 0·27 of a division for a *fall* in temperature of 1° C. The corresponding figures for distilled water and the copper sulphate solution are 0·20 and 0·25.

The copper sulphate method has considerably less value when the milk is sour, as the refraction obtained as the milk becomes sour is at first increased and then, as decomposition continues, lowered. The acidity in terms of ml of 0·1 N sodium hydroxide per 10 ml of milk should be determined at the same time as the refraction. The refractions of samples showing greater acidities than 2·0 are of doubtful accuracy, but for acidities between 3·0 and 8·0 a rough correction may be made by subtracting 0·2 from the observed reading for each 0·1 of acidity above 2·0.

Calcium chloride serum. 30 ml of milk are thoroughly mixed with 0·25 ml of a solution of calcium chloride (sp. gr. 1·1375) in a tube, which is then closed with a cork through which is passed a short piece of glass tubing to act as a condenser. The tube is heated in a boiling water-bath for 15 minutes and then placed in cold water. Any water condensed in the tube is added and the serum decanted and filtered.

Phosphotungstic acid serum. 7 g of phosphotungstic acid are dissolved in water, 2·5 ml of concentrated hydrochloric acid added, and the whole diluted to 100 ml. The refraction of the resulting solution should be adjusted to exactly 36·00 at 20° C, if necessary, by the addition of water or hydrochloric acid. To prepare the serum, 20 ml of milk are mixed with 5 ml of the solution, the whole well shaken and then filtered through paper.

Dialysed iron serum. Equal quantities of milk and dialysed iron are mixed together, thoroughly shaken and filtered through paper.

Sour serum. The milk is allowed to stand, preferably at 21° C (the temperature of the cool incubator), until the milk has coagulated sufficiently for a clear serum to be obtained on filtration. The preliminary thickening should be ignored, as it is not usually possible to obtain a clear liquid until coagulation is fairly complete.

In the preparation of this section full use has been made of a series of papers by Elsdon and Stubbs (1927), (1928a), (1928b), (1929), (1930a).

Detection of heated milk by determining refractive index

The determination of the refractive index of milk serum, besides being of some value for the detection of added water, might also possibly be adapted to the detection of the presence of heated milk. A certain amount of unpublished work was carried out by Elsdon and Heron in order to investigate this point, and they found that there is a fairly constant difference of about 2·0 units in the reading on the immersion refractometer scale of the copper serum of a milk determined before and after the milk has been heated to 100° C. This difference is probably due to the fact that, by heating, the albumin has been converted into a form which is precipitated by copper sulphate, and therefore the difference is probably a measure of the amount of soluble albumin present.

Sterilised milk shows no difference in the refraction before and after heating, due to the fact that sterilised milk has, of course, already been heated. On the other hand, pasteurised milk, by this test, behaves like raw milk, as the temperature of pasteurisation is not high enough to alter the albumin.

The change in the albumin appears to take place at about 70° C, and the following table indicates the results obtained by the above method by heating a sample of milk for different lengths of time at this temperature and at 100° C—

Table 5.12—Difference in the immersion refractometer readings of raw and heated milk (copper serum)

Temperature and time	Refraction before heating	Refraction after heating	Difference in refraction
Till just on 70° C ..	38·2	37·4	0·8
70° C for 5 mins. ..	38·2	37·35	0·85
,, 15 ,, ..	38·2	37·22	0·98
,, 30 ,, ..	38·2	36·65	1·55
,, 45 ,, ..	38·2	36·6	1·6
100° C for 15 mins. ..	38·2	36·25	1·95

From the above table it appears that after heating at 70° C for 30 minutes approximately 79 per cent of the albumin has been rendered insoluble. On the other hand, according to Rupp (1913) 30·78 per cent coagulates on heating at 71·1° C for 30 minutes. In order to find which of these figures is nearer the truth, and at the same time to investigate the possibility of the immersion refractometer being used as a rapid method for the determination of albumin in milk, further work is required to compare results obtained by this method with the results obtained by Moir's method for albumin (p. 365) on portions of the same milk, before and after they have been subjected to varying amounts of heat treatment.

(3) THE FREEZING POINT OF MILK

As will be shown later, the average (Hortvet) freezing point of milk is about —0·545° C. It is not, therefore, very different from that of water, so that if use is to be made of this determination it is necessary to find a method—not too laborious—which will give results correct to a few thousandths of a degree. Cryoscopy has made rapid strides within the last sixty years, and it is now possible to measure temperatures near the freezing point of water to the above degree of accuracy by means of mercury-in-glass thermometers and to less than 0·001° C by means of platinum-resistance thermometers.

In the year 1714, Fahrenheit discovered that water can be supercooled without freezing, and that if then a small particle of pure ice be added freezing commences, and the temperature of the system rises exactly to zero. From this observation sprang modern methods of determining the freezing points of dilute solutions. In the year 1788, Blagden, working on the already known fact that the freezing point of a solution was lower than the freezing point of the pure solvent, discovered that the lowering of the freezing point (dilute solutions) was proportional to the amount of solute present.

It is a well-known fact that when a dilute solution freezes, the solid phase which separates consists of the pure solvent. This solid phase is not, therefore, in equilibrium with the original solution but with a slightly more concentrated one, as the amount of the solute present is now dissolved in an amount of solvent less than that originally present by the amount that has been deposited as the solid phase. The freezing point determined is, therefore, not that of the original liquid. It is usually described as the " observed " or " uncorrected " freezing point. It is possible by the application of suitable corrections to obtain the " true " freezing point. The method of obtaining this correction, called the " supercooling correction ", is dealt with below. Apart from the correction necessary on account of supercooling, another error may be introduced by the cooling bath being at too low a temperature.

The present satisfactory position of cryoscopy in general is due largely to the work of Raoult, who published his first paper in the year 1878 and continued to publish work on this subject almost up to the time of his death in 1901. As a result of his long experience he developed a cryoscope of great accuracy, and worked out the necessary corrections to be applied to observed readings in order to obtain " true " freezing points. His observations on the freezing points of solutions of cane sugar are regarded as standards up to this day.

The cryoscope due to Raoult has been improved by Monier-Williams (1914). He introduced the Dewar flask as an insulating medium, and used ether, through which he passed a current of air, as the cooling medium. This is probably the most accurate form yet devised, but, as stated by Monier-Williams, it is unsuitable for routine work; he later devised a type more suitable for this purpose. As they are only used for special purposes, the more complicated types of cryoscope will not be described here. They are fully described by Monier-Williams (1933) and by Raoult in his book entitled *La Cryoscopie*, published in 1901, and in his published papers.

In the preparation of this chapter full use has been made of papers by Stubbs (1935a), Elsdon and Stubbs (1930b), (1933), Stubbs and Elsdon (1934), (1936). The revisers also wish to acknowledge their indebtedness to the reports on the subject made by Stubbs to the Lancashire County Council (1932), (1934), (1935b).

The true freezing point of solutions

Indication has already been made that the observed reading for the freezing point of a solution may be subject to various corrections if the " true " freezing point is to be obtained. Some workers have raised the objection to the Hortvet cryoscope (*vide infra*) that it does not indicate true freezing points; but, of course, precisely the same objection applies to the cryoscopes of Raoult and of Monier-Williams and, indeed, to all cryoscopes the application of which depends upon the principle, introduced by Rüdorff, of supercooling.

When such supercooling takes place and crystallisation is induced by the introduction of a small particle of the solid solvent, only pure solvent crystallises out, and the concentration of solute in the liquid phase is increased. The observed freezing point is, as we have seen, not that of the original solution but one slightly more concentrated—the amount of concentration being measured by the amount of solid solvent crystallising out.

It has been shown by Raoult that the effect of supercooling on the freezing point may be expressed by the formula $C' = C + KCS$, where C' is the observed depression of the freezing point, C is the true depression, S is the amount of supercooling, and K is a constant, determined experimentally, which is slightly different for different solutions but which is of the order of 0·015. This formula is an abbreviated one, the full formula from which it is derived being—

$$C = C' - \frac{CS}{L}\left(1 + \frac{r}{R}\right),$$

where C, C' and S have the same significance as before; L is the latent heat of the solvent; r is the water value of the instrument, and R the water value of the solution. As pointed out by Stubbs (1935) the larger formula only applies when the specific heat is unity and when, therefore, R represents both the weight and the water-value of the solution. The more general case can be represented by the formula:

$$C = C' - \frac{CS}{L}\left(H + \frac{r}{W}\right),$$

where W is the weight in grams of a solution whose water value is R and H is the specific heat of this solution.

The constant K is the fraction of the true depression by which it is increased for each degree of supercooling, when the solution remains in thermal equilibrium with its surroundings. When the freezing-point tube of a cryoscope is at such a temperature that it is in thermal equilibrium with the rest of the cryoscope, such a temperature is called the *convergence temperature*. This depends upon the design of the cryoscope and on the temperature of the cooling bath. It is determined by experiment.

The observed freezing point depends not only upon the extent of supercooling but also upon any net transference of heat which takes place during the actual crystallisation. This second correction becomes zero when the freezing point of the solution is identical with the convergence temperature. When the freezing point is not taken at the convergence temperature, the correction to be applied for this source of error is given by Raoult as:

$$\frac{C}{L}\left(1+\frac{r}{R}\right)\frac{y}{z},$$

where, in addition to the previous symbols, y is the time which elapses from the commencement of the freezing until the temperature has become nearly stationary, and z is the time necessary for the freezing-tube and contents to change one degree by heat transference at the temperature of freezing but without formation of ice. The correction is positive if heat is abstracted, and negative if heat is added. For a solution of which the specific heat is not unity the equation becomes, using the notation as before—

$$\frac{C}{L}\left(H+\frac{r}{W}\right)\frac{y}{z}.$$

Raoult's full equation for the corrections to be applied thus becomes—

$$C' = C + \frac{CS}{L}\left(H+\frac{r}{W}\right) + \frac{C}{L}\left(H+\frac{r}{W}\right)\frac{y}{z}.$$

When a similar solution is used in the same cryoscope, H, W, r, L and z are constant and y will be approximately constant, so that the expression then becomes—

$$C' = C + KCS + K'C;$$

and where the freezing point is at the convergence temperature of the apparatus,

$$C' = C + KCS,$$

which is the simplified correction for supercooling.

In addition to these two corrections there are others. Thus, apart from the errors inherent in a mercury thermometer, we have the " lag " produced by the very fine capillary. This can be overcome by gently tapping the top of the thermometer with a small hammer made of suitable material. There is also error due to the emergent stem. This can, of course, readily be allowed for, but it is not material in the case of the Hortvet apparatus, providing that all determinations are made at approximately the same laboratory temperature.

Determination of the freezing point of milk

Dreser (1892) found that the osmotic pressure of cows' blood was constant or nearly so; his results were confirmed by Hamburger, Bugarszky and Tangl, von Koranyi, Strauss, Koeppe and others, whilst some of these investigators (Dreser appears to have been the first) also reported the constancy of the freezing point of milk. Some years later, Beckmann and Jordis (1895) suggested that the method could be used for the detection of added water in milk.

Most of the early investigators seem to have used the type of cryoscope due to Beckmann which has since become so familiar as a method of determining molecular weights. No corrections seem to have been applied by the majority of workers up to the time of Monier-Williams, which doubtless accounts for the somewhat wide variations which are found in the literature.

In 1914 Monier-Williams published his now classical report on the subject. At the conclusion he states that " the freezing point appears to be the most constant of any of the properties exhibited by genuine milk. Although unaffected by the removal of fat from, or the addition of separated milk to genuine milk, it is raised by the addition of water to the milk. The method may, in certain circumstances, be applied with advantage, as a confirmatory test, to the detection of added water and to the approximate estimation of the amount present. Owing, however, to the experimental difficulties involved in obtaining reliable results, it is somewhat doubtful whether the method is capable of general application for purposes of milk control."

It may be that this final sentence was taken more seriously than was intended, as, at the time, no one seems to have followed up the suggestion contained in the Report for the simplification of the experimental technique. Thus Monier-Williams states that the difficulties could be very much reduced, if not wholly eliminated, by dispensing altogether with the determination of the zero point and comparing the observed freezing point of milk with that of an appropriate solution of cane sugar. If the two determinations are carried out in precisely the same manner, they will both be affected to a similar extent by the errors mentioned above and any due to the thermometer; and the difference between the results so obtained will indicate fairly accurately the true freezing point of the milk. If this mode of working be adopted, he said, it should be possible to carry out freezing-point determinations on milk samples without undue expenditure of time and with a degree of accuracy very little inferior to that attained by the use of more complicated apparatus.

No one then appears to have taken advantage of this valuable suggestion, but in 1917 Hortvet and his collaborators commenced work under the auspices of the American Association of Official Agricultural Chemists. In the resultant paper Hortvet (1921) stated that " there has doubtless been a lack of uniformity as regards the conditions under which the freezing-point determinations have been carried out . . . there does not appear to be any fairly defined uniformity in respect of design and construction of apparatus, and even in cases in which the ordinary Beckmann cryoscope has been used there are notable differences as regards the conditions under which determinations have been carried out ". Hortvet endeavoured to devise a cryoscope in which the application of correction factors might for all practical purposes be avoided by means of a carefully standardised method of procedure. Such an instrument was described: it is now widely known as the Hortvet cryoscope. It may be that Hortvet considered that his standardised technique gives, by a compensation of errors, a figure representing the true freezing point of milk, but subsequent work has shown that this is not the case and that the depression obtained is about 0·015° too large. As stated by Monier-Williams (1933) and others, suggestions have been made at various times that the Hortvet figures should be corrected so as to represent more nearly the true freezing point of milk, but these suggestions have not usually been adopted, owing, maybe, to the uncertainty as to the magnitude of the corrections to be applied. It has been considered preferable to adhere to the apparent freezing point as determined by a standard cryoscope, rather than to confuse matters by attempting to apply corrections, which may or may not be themselves correct. To endeavour to apply corrections to Hortvet readings shows a lack of understanding of the reasons which caused Hortvet to design the particular instrument which bears his name.

The Council of the Society of Public Analysts (1933a) reported that " The Council has reviewed the merits of the various forms of apparatus at present available and the procedures employed therewith, and recommends—

> " (1) That for administrative purposes the freezing point of samples of milk should be determined in accordance with the Hortvet technique exactly as described in *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*, 3rd edn., 1930.
>
> " No correction, other than those directed therein, should be applied.
>
> " (2) That the freezing point thus obtained should be recorded, for example, as: Freezing point (Hortvet) —0·550° C."

Although Monier-Williams considered the use of a standardised cryoscope without corrections to be quite sound, he made an attempt to elucidate the nature and magnitude of the corrections which must be applied if it is desired to ascertain true freezing points; and as his work suggested that it was not easy to determine the corrections for the Hortvet cryoscope, he devised a simpler apparatus made of thin sheet metal and insulated with cork which, he suggested, was as easy to manipulate as the Hortvet, and by which the necessary corrections could be determined with considerable accuracy. By the use of the later Monier-Williams apparatus a determination takes longer and, as the heat insulation is far from perfect, the amount of ether used is very much greater. Apart from this, it was pointed out by Stubbs (1935a) that although the heat transference factor is much less in the Monier-Williams cryoscope than in the Hortvet yet it is not zero. The Hortvet cryoscope itself was modified along the lines of the Monier-Williams cryoscope (Elsdon and Stubbs 1934, Stubbs 1935a), but these modifications offer no material advantage for routine purposes over the standard Hortvet instrument, although the final modification of the Hortvet apparatus due to Stubbs appears to offer manipulative advantage over that of Monier-Williams. None of these writers has suggested that the new devices have any advantage over the original Hortvet cryoscope for administrative purposes. A normal milk examined in the Hortvet cryoscope gives a depression about 0·015 °C greater than the true depression.

The Hortvet cryoscope

The Hortvet cryoscope has been chosen for extended description in preference to any other because it is convenient, has been widely used, and can give results which are easily reproducible either in the same or in different laboratories. Elsdon and Stubbs (1934) give the following results (Δ) as having been obtained by the examination of portions of the same sample of milk in six different laboratories widely separated throughout England: 0·549°, 0·551°, 0·552°, 0·549°, 0·551°, and 0·549° C. The fact that the results obtained are not " true " freezing points does not affect in any way the utility of the test, as they are always compared with the results obtained under precisely similar circumstances from genuine milks. If corrections *were* applied, a corresponding correction would be applied to each, so that the conclusions drawn would be identical.

The official directions for carrying out the test as given by the American A.O.A.C. are printed on pages 327 to 329. It is desirable, however, to discuss the more important parts of the apparatus and the method in the light of the experience of various workers.

The Dewar flask. Some observers have reported difficulty in obtaining Dewar flasks of the correct size. The total capacity should be 1,000 ml (suggested tolerance ± 50 ml) and the internal depth 280 mm (± 5 mm). The flask should have cylindrical walls from the base upwards, and there should be no constriction at the neck.

Stirring

Many observers have drawn attention to the labour involved in the working of the stirrer by hand and have devised methods for carrying it out by means of a small motor; such appliances may now be obtained commercially. When using such devices, however, they should be disconnected at the onset of freezing and the determination completed by hand, stirring exactly as set out in the official instructions.

Monier-Williams has pointed out that in the Hortvet method more efficient stirring may lead to erroneous results, on account of the difference due to greater heat transference between the freezing-tube and the cooling-jacket, although most observers have found that with the Hortvet type of stirrer small variations in the amount of stirring have little, if any, effect on the results. Stubbs (1935a) has even shown that the substitution of a more efficient type of stirrer has little influence on the result obtained, although it considerably reduces the time required for the taking of a reading from the onset of stirring. It must be emphasised, however, that for normal work the Hortvet stirrer should be used and in the manner recommended by him. When the freezing point is determined, it occasionally happens that the mercury only remains at the highest point for a sufficient time for a rough reading to be taken. Such experiments should be discarded, as it has been found that these, even when a reading can be taken, usually give too great a depression.

The cooling process

In the original apparatus the reduction in temperature of the ether in the cooling-bath is carried out by means of a hand blower. This hand blower can conveniently be replaced by an electrically operated blower, controlled by means of a switch placed on the right-hand side of the apparatus. In place of blowing, a current of air may be drawn through the apparatus by means of a water-pump, the cooling-bath having been carefully sealed. When the blowing method is used, the ether vapour may be led into the outside atmosphere by means of a suitable tube of fairly large bore.

The thermometers (Stubbs and Elsdon 1936)

The freezing-point thermometers usually supplied with the Hortvet apparatus are of mercury-in-glass, graduated from + 1·0° to —2·0° C, the length of one degree being about 10 cm. The primary standards for Hortvet's work were two thermometers standardised by the American Bureau of Standards to an accuracy of from ± 0·002° to ± 0·005°, although the corrections were stated to 0·001°. These two thermometers were used to find in the Hortvet apparatus the freezing points of solutions made by dissolving 7 and 10 grams respectively of pure cane sugar (obtained from the Bureau of Standards) in water and making each solution to 100 ml at 20°. Under the conditions of working, the differences between the freezing points of water and the solutions were found to be 0·422° and 0·622° for the 7 and 10 per cent wt./vol. solutions, respectively, for one thermometer, and 0·422° and 0·621° for the other. The interval of 0·199° was accepted as the interval between the freezing-depressions of 7 and 10 per cent wt./vol. sugar solutions.

Exactly the same procedure was followed for other thermometers and, if necessary, a factor was applied to the readings to convert the differences between the freezing-point depressions of the two sugar solutions to 0·199° C. This factor was then applied to all readings of freezing-point depression obtained by using the same thermometer, apparatus and procedure.

The A.O.A.C. suggest the standardisation of Hortvet thermometers at two definite points, viz. —0·422° C and —0·621° C. But as neither of these two temperatures is one which is met with in the examination of genuine milk, it is necessary to standardise at intermediate temperatures. The A.O.A.C. suggest that this be done in the following manner—

The thermometer is checked against each of the sucrose solutions. The freezing-point depression of each of these two solutions is taken in turn, using the thermometer to be tested. The interval between the two readings obtained is compared with the standard interval of 0·199° C. Thus, in the case of a thermometer in which the interval found was 0·205° C (readings of —0·420° and —0·625° respectively), the correction for a reading of 0·548° would be obtained by the expression (0·548° — 0·420°) 0·971[1] = 0·124°, and, corrected, the depression would therefore be 0·422° + 0·124 = 0·546° C.

This method of examination assumes that any error in a thermometer between the two points corresponding with —0·422° and —0·621° will increase or decrease uniformly in proportion with the graduation marks of the thermometer from —0·422° to —0·621° C. This supposition is most unlikely to be true, as it depends on equidistant spacing of the graduations, and also on the uniformity of the bore of the capillary tube between these two points. Errors may arise from either or both of these causes, and it is necessary to fix more definitely some of the intermediate points.

As the Hortvet sugar solutions are made up by weight in volume of solution and not by weight in weight of solvent, the freezing-point depressions of solutions of intermediate strengths will not be exactly proportional to the quantity of sugar dissolved, but the error involved will not be large, as the standard is fixed at both ends of the scale. Whilst for the purposes of the ordinary examination of milk an error in the thermometer of the order of ± 0·002° C is of no vital importance, it is desirable to fix the intermediate points more closely than this.

In order to calculate the freezing-point depressions of sugar solutions of intermediate strengths between 7·0 per cent and 10 per cent wt./vol. by means of Raoult's formula, it is necessary to know the amount of sugar dissolved in 100 g of water in each case. This figure was carefully determined, on the seven sucrose solutions concerned, by weighing out the necessary amount of sugar, transferring it to a clean and tared 200-ml graduated and calibrated flask, dissolving the sugar in water, and making up to the mark after the flask had been allowed to stand in a thermostat at 20° ± 0·1° C for one hour. The flask and contents were weighed, and the weight of sugar dissolved in 100 g of water was calculated in each case. The following results were obtained—

---

[1] Interval on thermometer under test = 0·205, standard interval 0·199; therefore the factor becomes $\frac{0{\cdot}199}{0{\cdot}205} = 0{\cdot}971$.

Table 5.13—"True" freezing-point depressions of Raoult's sucrose solutions

Grams of sucrose in 200 ml of solution	Grams of water in 200 ml of solution	Grams of sucrose in 100 g of water	Δ° C (calc. from Raoult's formula)
14·0	190·83	7·336	0·4103
15·0	190·22	7·886	0·4417
16·0	189·60	8·439	0·4735
17·0	188·97	8·996	0·5056
18·0	188·35	9·556	0·5379
19·0	187·75	10·120	0·5706
20·0	187·15	10·687	0·6037

A similar determination has been made by Monier-Williams for the 7 per cent and 10 per cent wt./vol. solutions, results of 7·3373 and 10·6895 being obtained. These two different determinations are in close agreement, the difference only just affecting the fourth place of decimals in the freezing-point depression.

The 7·336 per cent wt./wt. solution (7·0 per cent wt./vol. Hortvet) was found experimentally by Hortvet to have a freezing-point depression, in his cryoscope and by his technique, of 0·422° C and this figure has been adopted by the A.O.A.C. as one of the standards with which all similar thermometers are to be compared. For the 10·687 per cent wt./wt. solution (10·0 per cent wt./vol. Hortvet) the corresponding figure is 0·621° C. When the freezing-point depressions of these two solutions are calculated by means of Raoult's formula, viz.—

$$\text{F.P.D.} = \frac{18{\cdot}72 \times P}{342-(0{\cdot}99 \times P)},$$

where P = the concentration of the sucrose solution expressed in grams of sucrose per 100 g of water, the figures obtained are 0·4103° C and 0·6037° C respectively. There is thus a difference between the Hortvet figure and the Raoult figure of 0·0117° C at —0·41° C and of 0·0173° C at —0·60° C.

These differences are, of course, caused by the fact that the Raoult figures are " corrected ", whilst the Hortvet figures are " uncorrected ".

There is good reason to suppose that the difference between the two readings (Hortvet's and Raoult's) will be more or less proportional to the depressions, so that, at least as a first approximation, if we assume 0·0117° C as the difference at 0·41° C, then the difference at 0·60° C may be expected to be—

$$\frac{0{\cdot}0117^\circ\ \mathrm{C} \times 0{\cdot}6037}{0{\cdot}4103} = 0{\cdot}0172^\circ\ \mathrm{C}.$$

The actual difference is 0·0173° C, so that the agreement is extremely close and is valuable evidence of the mutual concordance of the two results obtained by Hortvet as his reference temperatures.

Having fixed the two extremes, it is possible to calculate the Hortvet freezing-point depressions of the intermediate solutions. The results obtained are shown in the following table—

Table 5.14—Freezing-point depressions (Hortvet) of sucrose solutions

Strength of sucrose solutions		Freezing-point depression (Raoult) °C	Calc. Hortvet		Suggested Hortvet standard freezing-point depression °C
Grams per 100 ml of solution	Grams per 100 g of water		Correction to be added °C	Total °C	
7·0	7·336	0·4103	0·0117	0·4220	0·422
7·5	7·886	0·4417	0·0126	0·4543	0·454
8·0	8·439	0·4735	0·0135	0·4870	0·487
8·5	8·996	0·5056	0·0144	0·5200	0·520
9·0	9·556	0·5379	0·0153	0·5532	0·553
9·5	10·120	0·5706	0·0163	0·5869	0·587
10·0	10·687	0·6037	0·0173	0·6210	0·621

It will be observed that very little correction is necessary to round off the figures to three places of decimals. In order, therefore, that the method of standardisation should be as uniform as possible, the figures given in the last column are suggested as standard Hortvet reference-points at the intermediate temperatures. If, for any purpose, further intermediate temperatures are required, solutions of suitable strength may be prepared, and their freezing-point depressions calculated by simple proportion from any two adjacent pairs of figures, as any error involved is negligible.

It appears to be rather more difficult to obtain concordant readings for the freezing points of sugar solutions than for milk. Thus, whilst it is very unusual for duplicate determinations of the freezing point of milk to differ by more than 0·001°, solutions of cane sugar not infrequently show differences up to 0·005°. By making a sufficiently large number of determinations a good average figure can be obtained, but it is suggested that it might be advisable to consider the possibility of using solutions of pure sodium chloride or sugar solutions made up with a dilute solution of some colloid, as it may be that the colloidal nature of milk protects it during the freezing process from the more obvious effects of heat transference. This might account for the differences noticed between milk and solutions of cane-sugar.

The zero of the thermometer should be determined at least once each day as, even when it has settled down, changes in barometric pressure cause an appreciable difference in the results obtained.

A suitable thermometer has a total length of 580 to 600 mm.; the length of one degree on the scale should be 90 to 100 mm. The diameter of the stem should be 6·5 to 7·0 mm, that of the bulb 9·5 to 10·0 mm, and the length of the bulb from 55 to 65 mm. The bulb should be cylindrical and the safety bulb at the top of the thermometer should consist of one compartment only, which should be in the shape of an inverted pear.

To carry out the actual reading, it is convenient to use a small hand lens about 20 mm. in diameter and with a focal length of about 45 mm. With such an aid it is usual for two observers to make identical readings of the same position of the mercury. For those who find difficulty in avoiding errors due to parallax the " magnifying reader " can be recommended (Lockwood 1932, Elsdon and Stubbs 1933a).

The control thermometer

The control thermometer specified by the A.O.A.C. is of the same external size as the freezing-point thermometer, but has a range of from —30° to + 20° C. This range is unnecessarily large and may with advantage be decreased. A suitable thermometer is one with a solid stem approximately 580 mm in length, 6·5 to 7·0 mm in diameter, and having a scale range of —5° to + 5° divided into fifths of a degree. The safety bulb should be of the same character as that of the standard thermometer.

Temple (1937) has designed an apparatus which, whilst retaining the satisfactory principles involved in the Hortvet apparatus, should reduce the manipulative difficulties and the cost. The essential principle of the apparatus is that the cooling of the milk takes place in a bath of calcium chloride solution of such a concentration that it freezes at —2·3°, which temperature is maintained by circulated brine thermostatically controlled at —5° by means of a sulphur-dioxide compressor unit. The whole apparatus is so arranged that it can be readily moved from one laboratory to another, and that it requires only to be plugged-in to the electric supply to be ready for service. The results obtained are claimed to be the same as those given by the Hortvet apparatus, and that the probable error of a single determination is less, viz. 0·001° as against 0·0014°. This apparatus appears to offer many advantages when it is necessary to make large numbers of determinations.

Variations in the freezing point of milk

A vast number of results have now been published for the freezing point of genuine milk. In many of these, particularly in the older observations, no mention has been made of the corrections which have been applied. In most of these, however, the average result obtained has been of the order of —0·55° C, so that it can be assumed with much justification that corrections have not been applied. The extreme range which has been recorded is from —0·490° by Atkins to —0·748° by Tocher, but later experience would suggest that the lower of these temperatures was that of a sour milk. The figures published by many observers are collected together in Table 5.15.

Table 5.15—Freezing-point depressions of milk (° C) (*various observers*)*

Observer	Average	Ordinary range	Extreme range
Dreser	—	0·55 to 0·57	—
Winter	0·555	0·55 ,, 0·56	0·54 to 0·57
Bordas and Génin	—	0·512 ,, 0·529	—
Henderson and Meston (1913)	0·550	0·54 ,, 0·56	—
Henderson (1929)	—	0·545 ,, 0·55	0·545 to 0·565
Monier-Williams	0·537	0·53 ,, 0·55	0·519 ,, 0·558
MacLaurin	0·550	—	0·545 ,, 0·565
Ducros and Imbert	—	—	0·533 ,, 0·575
Hummelinck	—	—	0·542 ,, 0·570
Van Raalte	—	—	0·540 ,, 0·570
Keister	—	—	0·541 ,, 0·574
Gooren	—	—	0·530 ,, 0·570
Atkins	0·550	0·52 to 0·58	0·490 ,, 0·610
Stoecklin	—	—	0·545 ,, 0·565
Leather	—	—	0·529 ,, 0·577
Reicher	—	0·550 to 0·580	0·541
Joseph	—	—	0·538 to 0·579
Bolm	0·550	—	0·530
Gronover and Türk	—	0·540	—
Koenig and Kluge	—	0·540	—
Klamer	—	0·53 to 0·55	0·52 to 0·56
Fiehe and Kordatzki	0·552	0·537 ,, 0·576	—
Andrew	0·555	0·550 ,, 0·560	0·545 to 0·565
Buchanan and Lowman	—	—	0·537 ,, 0·582
Filippo	—	0·53 to 0·54	—
Beckmann	0·554	0·54 ,, 0·58	—
Hamburger	—	0·556 ,, 0·574	—
Carlinfanti	—	0·55 ,, 0·59	—
Bomstein	—	0·540 ,, 0·570	—
Cornalba	—	0·55 ,, 0·56	—
Stubbs and Elsdon	0·544	0·533 ,, 0·555	0·529 to 0·563
Denis-Lester	0·541	—	0·528 ,, 0·561
J. Krenn	0·545	—	—
K. Wiss	0·545	—	—
R. Bauer	—	—	0·526 to 0·570
G. Buogo	0·570	0·562 to 0·575	—
M. Saito†	0·555†	—	0·555 to 0·580†
H. A. Schuette and E. O. Huebner	0·550	—	0·532 to 0·563
H. Alfonsius	0·543	—	0·532 ,, 0·554
H. Svoboda	0·540	0·535 to 0·545	—

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* It should be remembered that with freezing points lower than zero, the smaller number is the higher freezing point. As some little confusion is likely to arise from this, there is a distinct advantage in using the depression of the freezing point below that of water—usually denoted by Δ. This latter method obviates the use of the negative sign. The terms "higher" and "lower" should be used for freezing points, and "greater" or "less" for depressions.

† Different communications.

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The most carefully determined of all figures so far published are probably those of Monier-Williams. His figures are, however, corrected and indicate the true freezing points. He obtained an extreme range of from —0·519° to —0·558°, with an ordinary range of from —0·53° to —0·55° and an average of —0·537° C. These figures can be compared with Hortvet results by increasing the depression by about 0·016°, so that they then show an extreme range of from —0·535° to —0·574° with an average of —0·553° C.

Hortvet in 1919–1920 examined sixty milks from individual cows and fifteen from herds, with the following results—

Table 5.16—Freezing-point depressions of milk (*Hortvet*)

	Individual cows			Herds		
	Fat per cent	Solids-not-fat per cent	*Δ* °C	Fat per cent	Solids-not-fat per cent	*Δ* °C
Maximum	7·30	10·15	0·562	5·50	9·27	0·562
Minimum	2·80	7·37	0·534	3·10	8·48	0·545
Average	4·06	8·93	0·547	4·15	8·95	0·551

Bailey and his collaborators (1922) examined the milk of 179 normal individual cows, 61 herds, and 37 diseased or otherwise abnormal herds, and gave 11 other unclassified results requiring further corroboration. The normal results are given in the following table—

Table 5.17—Freezing-point depressions of milk (*Bailey*)

	Individual cows			Herds		
	Fat per cent	Solids-not-fat per cent	*Δ* °C	Fat per cent	Solids-not-fat per cent	*Δ* °C
Maximum	7·3	10·26	0°566	5·5	9·30	0·562
Minimum	0·9	7·20	0·530	2·7	7·98	0·530
Average	3·8	8·71	0·545	3·8	8·58	0·544

Of the milks from abnormal or diseased cows only 80 per cent had depressions within the limits observed for healthy cows; the others gave greater depressions. Only one animal gave milk having a depression of less than 0·530°, namely —0·523°. This was the milk from an individual cow. Three further samples were obtained from the same cow, but a similar low result was not obtained. Later, Bailey (1923) found that tubercular cows gave depressions within the normal limits, even after the animals had been subjected to the tuberculin test. The tendency of the test was to increase the depression slightly.

Following up the abnormal results which he had obtained in the previous year (all of which were from the same herd), Bailey examined 40 samples from 19 individual cows of the herd, with the following results: maximum 0·568°, minimum 0·532°, average 0·547°, herd average 0·554° C.

Tocher (1925) determined the depression of the milk of 676 individual cows. The technique of Hortvet was not, however, followed in its entirety, although the same apparatus was used. The supercooling was carried out to the extent of about 2·0°, and the Raoult correction for this was applied. The Raoult correction for 2·0° supercooling for a depression of 0·548° is 0·018°, so that Tocher's average for the observed readings becomes 0·566°, which is very considerably higher than that of other observers. This high average is apparently due to the fact that some of the samples were slightly sour. Tocher's minimum corrected figure was 0·505°, which becomes 0·522° when the appropriate correction factor has been returned.

Stubbs and Elsdon (1934) examined a total of 1,000 samples of milk. Of these 330 were "appeal-to-cow" samples; the others were considered to be genuine, but no figure was excluded unless a subsequent sample from the cows had given one materially greater than the one excluded. Tables 5.18 to 5.21 have been taken from this paper—

Table 5.18—Freezing-point depressions: all samples

	"Appeal-to-cow" samples	Other samples	All samples
No. of samples ..	330	670	1000
Average	0·545	0·544	0·544
Extremes	0·529 0·563	0·530 0·563	0·529 0·563

Table 5.19—Freezing-point depressions: morning and evening milks

	Morning samples	Evening samples	Unknown	Total
No. of samples	440	500	60	1000
Average ..	0·545	0·544	0·544	0·544
Extremes ..	0·530 0·563	0·529 0·563	0·530 0·556	0·529 0·563

It will be seen from Table 5.21 that the depression of the freezing point appears to be quite independent of the percentage of solids-not-fat. This would be expected, as the depression depends upon the osmotic pressure and not on the total weight of solids-in-solution. A deficiency or surplus in one ingredient of the solids-not-fat, usually lactose, is balanced as regards osmotic pressure by an increase or decrease in another constituent, e.g. mineral salts, and a small amount of the latter has the same effect in depressing the freezing point as a much larger amount of lactose.

Table 5.20—Freezing-point depressions: frequencies—morning and evening milks

Freezing-point depressions		Morning samples	Evening samples	Unknown samples	Total samples
0·529	0·530	2	3	1	6
31	2	2	2	—	4
3	4	10	9	1	20
5	6	14	21	1	36
7	8	31	36	1	68
9	0·540	43	67	11	121
0·541	2	53	61	11	125
3	4	62	83	12	157
5	6	68	65	6	139
7	8	48	55	6	109
9	0·550	39	39	5	83
0·551	2	23	26	2	51
3	4	11	21	2	34
5	6	13	9	1	23
7	8	7	1	—	8
9	0·560	9	1	—	10
0·561	3	5	1	—	6
		440	500	60	1,000

Table 5.21—Freezing-point depressions and solids-not-fat: all samples

Solids-not-fat per cent	No. of samples	Freezing-point depressions		
		Average	Least	Greatest
7·6 to 7·9	4*	0·545	0·535	0·555
8·0	6	0·544	0·537	0·552
8·1	5	0·546	0·529	0·559
8·2	22	0·543	0·536	0·550
8·3	36	0·543	0·533	0·552
8·4	62	0·543	0·529	0·559
8·5	95	0·544	0·531	0·563
8·6	103	0·543	0·530	0·557
8·7	128	0·544	0·533	0·563
8·8	156	0·543	0·530	0·561
8·9	147	0·545	0·533	0·561
9·0	116	0·547	0·533	0·563
9·1	61	0·546	0·532	0·559
9·2	37	0·545	0·531	0·556
9·3	9	0·548	0·545	0·556
9·4	8	0·546	0·538	0·560
9·5 and 9·6	5†	0·547	0·539	0·550
	1,000	0·544	0·529	0·563

* 1 each of 7·6, 7·7, 7·8 and 7·9 per cent.

† 3 of 9·5 per cent, 2 of 9·6 per cent.

Table 5.22—Freezing-point depressions: monthly variations

Month	No. of samples	Freezing-point depressions Average	Least	Greatest
January	51	0·543	0·530	0·559
February ..	123	0·543	0·530	0·561
March	78	0·544	0·531	0·559
April	70	0·546	0·533	0·563
May	82	0·544	0·535	0·563
June	69	0·546	0·535	0·560
July	116	0·545	0·535	0·559
August	77	0·545	0·533	0·556
September ..	62	0·544	0·534	0·557
October	114	0·545	0·529	0·561
November ..	91	0·544	0·529	0·555
December ..	67	0·544	0·530	0·559
	1,000	0·544	0·529	0·563

Table 5.23—Freezing-point depressions: June to September, 1933

Month	No. of samples	Freezing-point depressions Average	Least	Greatest
June, 1933 ..	35	0·545	0·535	0·560
July, 1933 ..	71	0·546	0·535	0·559
August, 1933 ..	53	0·545	0·533	0·555
September, 1933	29	0·544	0·530	0·557
	188	0·545	0·530	0·560

The weather in Britain during the months of June, July, August and September, 1933, was exceptionally dry and hot, and there was an unusually large amount of bright sunshine. It will be observed that the abnormal weather did not materially affect the freezing-point depressions.

Stubbs and Elsdon have examined statistically a very large number of freezing-point depressions obtained by the Hortvet process in different parts of England, Scotland and Wales. The results obtained are in agreement with those contained in Tables 5.18 to 5.23 above.

Elsdon and Stubbs (1931) stated that the Hortvet freezing-point apparatus could be confidently recommended as a convenient and rapid means for the determination of the freezing point of milk. The average depression for genuine milk might be taken as 0·54°, but no sample should be considered as watered on the evidence of the freezing point alone unless this fell distinctly below 0·53°. They considered that the results obtained would be of very considerable, if not conclusive value, in the detection of added water. Writing twenty years later, it only remains to be said that subsequent experience has entirely confirmed this opinion.

Denis-Lester (1933, 1936 and 1937) has determined the freezing-point depressions of South African milks, using the Hortvet apparatus and technique.

The range found was from 0·528° to 0·561° with an average of 0·541° C. It was found that period of lactation, season and diet had no apparent influence on the depression.

A few workers have reported occasional figures for the freezing-point depressions of genuine milk which are smaller than those usually accepted (Pawletta and Petersheyde 1934, Oestermann 1935).

Recent investigations on the freezing-point depression

A comprehensive study of the freezing-point test has been made by Aschaffenburg *et al.* Aschaffenburg and Temple (1941) have shown that morning milks tend to give higher freezing points, average values for the milks of three cows being —0·546, —0·546, and —0·544 (a.m.) and —0·548, —0·548, and —0·546 (p.m.) °C. Extreme values of —0·529 and —0·560 were found and there appeared to be a period of highest values from 14th May to 5th July. The coefficient of variation was less than 1 per cent whereas that for s.n.f. was 2·4 per cent. A general mean value of —0·548° C was obtained (1944a) and duplicates agreed to within 0·001° C. Plentiful young grass in spring led to high freezing points and warm temperatures to low. It was concluded that breed, but not yield, stage of lactation or age, may affect the freezing point. A slight correlation with solids-not-fat was observed. A survey of producer milks showed that 6 per cent contained added water but severe adulteration was exceptional, the mean adulteration of bulk supplies being less than 1 per cent (1944b).

A recent paper by Hillman, Provan and Steane (1950), has provided additional evidence on the F.P.D. of morning and evening milks. These workers claim that cows gave an evening milk having a markedly small depression, but the morning milk gave a rather large depression. They quote figures as follows for bulked milk in cans—

Evening milk ..	—0·540 to —0·519° C
Morning milk ..	—0·572 to —0·548° C

A normal depression would have been shown if the evening and morning milks had been mixed.

Similar results are quoted by Aschaffenburg and Rowland (1950) for a bulked herd milk—

	Herd housed at night	*Herd on pasture day and night*
Evening milk ..	—0·531 to —0·526° C	—0·553 to —0·542° C
Morning milk ..	—0·566 to —0·555° C	—0·546 to —0·543° C

It was found that when the animals were housed at night they were deprived of water for some sixteen hours. When they were let out they drank copiously and also consumed a large ration of mangolds later in the morning. It is suggested that the heavy intake of water during the morning might lead to an excessive secretion of water in the evening milk and vice versa. The mixed evening and morning milks gave normal freezing points.

The animals were afterwards left out on pasture day and night, with free access to water at all times. Within a week the freezing points of the evening and morning milks became normal and were of the same order.

Gutierrez (1943) has obtained an average value of —0·542 (—0·560 to —0·531) for cows' milk and —0·539 (—0·545 to —0·527) for carabao's milk (°C). Values have been reported from India (1941) as follows: cows' milk, —0·540 to —0·555, mean —0·546° C; buffaloes' milk, —0·530 to —0·560, mean —0·543° C.

Beckel (1931) has proposed a formula which allows the calculation of the freezing point from the chloride content and the refractometer reading (R.R.) of the copper sulphate serum. Tellmann and St. Daim (1949) propose the following formula—

$$100\,\Delta = 2(\text{R.R.}-15) + 0{\cdot}7 \text{ chloride per cent}$$

using a calcium chloride serum. They claim that this holds for genuine and watered milks.

A very valuable report, which summarises information on the Hortvet test, has been issued in Scotland (1945). This report stresses that the freezing point is the most constant of all the properties of milk, but recommends that there should be a right of appeal to the cow in view of the fact that on rare occasions the depression of genuine milk from a cow may be less than 0·530° C. The apparatus standardised by the U.S. Association of Official Agricultural Chemists is recommended, and the Temple modification accepted as giving similar results. The method is also applicable to pasteurised and sterilised milks. No correction for souring need be applied provided that the acidity does not exceed 0·18 per cent " lactic acid ".

The standardisation of the Hortvet thermometer is obviously the fundamental aspect of the freezing-point test. Aschaffenburg and Hall (1949) are of the opinion that freezing points of sucrose solutions at concentrations greater than 8 per cent are unreliable, and do not approve of the standard methods of calibration of the thermometer for this reason. They recommend that Hortvet thermometers should be standardised at a recognised institution at about + 0·05, 0, —0·4, —0·5, —0·52, —0·54 and —0·56° C. Sutton *et al.* (1950) have emphasised the advantages of adopting the Hortvet method as an arbitrary technique and conclude that the use of sucrose solutions is justifiable. They report the following values—

Per cent sucrose ..	7	7·5	8	8·5	9	9·5	10
Average depression, °C	0·4144	0·4457	0·4771	0·5095	0·5420	0·5752	0·6087
Standard deviation, °C	0·0007	0·0008	0·001	0·0005	0·0011	0·001	0·0013

They also discuss the effect of various factors on the accuracy of the thermometer and show that atmospheric pressure, atmospheric temperature and mechanical changes in the bulb during a day's run can affect the ice-point and hence, of course, all readings. Further, variations in the dimensions of the tubes and in technique, particularly in relation to the method of stirring, also have an influence. Moreover, the authors draw attention to certain advantages in not using an alcohol layer between the tubes, as adopted in the Monier-Williams apparatus.

Sutton and Markland (1950) have re-examined the original method of Hortvet, using sucrose solutions for standardisation, and have not confirmed the irregularities reported by Aschaffenburg and Hall. Using N.P.L. certificated thermometers they obtained depressions smaller by 0·005° and 0·013° C than when using sucrose solutions. Data obtained for sucrose solutions with these thermometers were not in agreement with Hortvet's figures.

Aschaffenburg and King (1951) have recently obtained good agreement between the " sucrose " and the " N.P.L. thermometer " methods and also between the Hortvet and the Hortvet-Temple cryoscopes. They have confirmed Stubbs and Elsdon's value of —0·520° C for 8·5 per cent sucrose solution, and also obtained reproducible results for 8·75 but not for 9·0 per cent sucrose solutions.

Koenig (1947) has shown that cows on a starvation diet may produce milk having a small freezing *depression* (less than 0·530° C), the small value being 0·48° C and the blood having correspondingly lower osmotic pressures. Change to a diet including fresh grass resulted in a sudden change to a large depression (up to 0·59° C), the values becoming normal in a week. Tankard and Bagnall (1944) report extremes of —0·541 and —0·559° C for herd samples and of —0·536 and —0·564° C for individual cow samples.

Bulk milk

The bulking of milk naturally affects considerably the reliability of the freezing-point test because the individual variations of single cow samples are eliminated. Macdonald (1948) has investigated the freezing point of bulk milk over a period of many years and finds that the normal variation is from —0·542 to —0·548 with an average of —0·544. Whereas with farmers' milks it is necessary to adopt a limit of —0·530 to allow for the individuality of the cow, with bulk milk a limit of —0·540 can safely be adopted, as bulk milk has never been found to have a freezing point above this (smaller depression). The definition of bulk milk is a rather controversial matter. The revisers suggest 1,000 gallons in view of the fact that tankers are usually at least of this capacity. It is recommended that —0·544 be taken as the mean and all added water calculated on this basis, but that 1 per cent be " allowed " and no action taken unless over 1 per cent is present. The following table gives percentages of added water corresponding to various freezing points—

Table 5.24—Effect of added water on freezing point of bulk milk

Freezing point depression °C × 1000	*Percentage added water*	*Freezing point depression* °C × 1000	*Percentage added water*
538	1·1	518	4·8
536	1·5	516	5·1
534	1·8	514	5·5
532	2·2	512	5·9
530	2·6	510	6·3
528	2·9	508	6·6
526	3·3	506	7·0
524	3·7	504	7·4
522	4·0	502	7·7
520	4·4	500	8·1

The effect of souring on the freezing point

All observers are agreed that as a milk becomes sour the observed depression increases. For this reason the test should always be carried out on fresh milk—the acidity should be less than that indicated by 2 ml of 0·1 N sodium hydroxide for 10 ml of milk to phenolphthalein.

Bailey has found that each increase of 0·1 ml of 0·1 N sodium hydroxide for 10 ml of milk corresponds with an increase in the depression of 0·0026° C. Tocher found an almost identical figure, but Stoecklin found 0·0042°, whilst Keister found 0·0032° under similar conditions. Parker and Spackmann (1929) found that Bailey's correction holds good where the amount of alkali required for 10 ml of milk varies between 1·9 and 6·3 ml of 0·1 N sodium hydroxide solution, but that a greater correction is necessary for an acidity figure below 1·9. With unknown milks it is impossible to say exactly what increase of acidity has taken place; hence the desirability, apart from the uncertainty of the correction, of examining milks when fresh. For small increases of acidity above 2·0, an approximate correction is to subtract 0·003 from the observed depression for each 0·1 ml over 2·0 of 0·1 N sodium hydroxide solution used for 10 ml of milk.

Evans (1936) has endeavoured to find the original freezing point of a badly decomposed milk. He determines (by the Government Laboratory method) the amount of lactose in the original milk and dissolves this in a known volume of a solution prepared from the ash in such a way that the phosphorus and calcium are thought to be in a similar condition (as far as the effect on the freezing point is concerned) as they were in the original milk. The procedure is as follows. A full analysis is made of the sour milk by the Government Laboratory process, and the figures for fat and solids-not-fat in the fresh milk are thus obtained. In addition to this, the ash and proteins are determined, and, by subtracting the sum of these from the non-fatty solids figure, the percentage of lactose in the fresh milk is calculated. Alternatively, the amount of lactose in the original milk could be found by determining that in the sour milk, and correcting the amount so found by the usual method of the Government Laboratory.

From the figures for fat and solids-not-fat the gravity of the original milk is obtained, and a quantity equivalent to 50 ml is weighed out, evaporated to dryness, and carefully ignited at a very low temperature to a black char. It is then taken up with hydrochloric acid,[1] again evaporated to dryness, and taken up with water. Excess ammonia is added, and the whole is again evaporated to dryness, the carbonaceous mass being rubbed down to a fine powder while still moist. The whole is then gently heated in the mouth of a muffle until all white fumes of ammonium salts have been driven off. The residue, which is slightly alkaline at this stage, is brought into a neutral condition by being taken up with 10 per cent acetic acid, evaporated to dryness, and heated in the water-oven for several hours until all traces of acetic acid have been removed. It is then taken up with about 30 ml of cold water, and the requisite amount of 0·1 N citric acid solution is added to bring the reconstituted serum to the correct acidity as indicated by the s.n.f. figure.[1] The calculated amount of lactose for 50 ml is also added, and the whole is made up to a volume of 50 ml, less the volume occupied by the fat. The freezing point is then taken.

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[1] Acetic acid cannot be used at this stage in place of hydrochloric acid, because the ignited calcium phosphate cannot be dissolved in the former, irrespective of the strength used.

By operating in this way on sixteen samples of milk which had been examined when fresh, Evans obtained results which were, in general, quite near the truth. In twelve cases the depressions were too small by amounts varying from 0·001° to 0·019° with an average 0·008°, whilst in four cases the depressions were too large by amounts varying from 0·002° to 0·008° with an average of 0·004°. The complete range was therefore + 0·008° to —0·019° with an overall average of —0·005° C.

The method does not yet appear to have had an extended trial, but seems likely to yield useful results.

The freezing point of cows' colostrum

Monier-Williams found for four samples of colostrum, depressions of 0·528°, 0·547°, 0·549°, and 0°544° C respectively, but these being corrected figures will be about 0·017° smaller than uncorrected Hortvet figures. Engel and Schlag (1924) found that the depression in some instances became as great as 0·605°, but these were not Hortvet figures.

Elsdon (1934b) found with the Hortvet apparatus, for two cows, the following figures—

Table 5.25—Freezing-point of cows' colostrum

COW NO. 1

Jersey cow—Bull calf born 1 a.m., December 19th, 1933.

Number	Day	Time	Fat per cent	Solids-not-fat per cent	Ash per cent	Protein per cent	Δ*
G.2528	1	7 a.m.	4·1	14·1	1·10	9·09	0·570
2529	1	2 p.m.	10·9	12·0	0·99	7·94	0·576
2530	1	5.15 p.m.	12·3	10·8	0·91	6·60	0·576
2534	2	7 a.m.	6·0	10·5	0·95	4·65	0·579
2535	2	2 p.m.	8·6	9·4	0·84	4·47	0·562
2536	2	5.30 p.m.	8·6	9·2	0·87	4·53	0·558
2541	3	7 a.m.	5·6	9·7	0·84	4·36	0·563
2542	3	2 p.m.	7·1	10·1	0·92	4·58	0·580
2543	3	5.30 p.m.	4·6	9·5	0·84	4·29	0·559

* Determined in the standard Hortvet apparatus.

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[1] In general the natural acidity of a fresh milk is proportional to the solids-not-fat content. The following may be taken as average values—

Solids-not-fat per cent	Acidity ml 0·1 N per 10 ml	Solids-not-fat per cent	Acidity ml 0·1 N per 10 ml
6·2	0·8	8·4	1·6
6·5	1·0	8·5	1·7
7·1	1·2	8·8	1·8
7·8	1·4	9·0	1·9

COW NO. 2

Friesian cow—Calf born dead 5 a.m., December 18th, 1933.

Number	Day	Time	Fat per cent	Solids-not-fat per cent	Ash per cent	Protein per cent	Δ*
G.2531	2	7 a.m.	1·3	21·9	1·20	17·46	0·590
2532	2	2 p.m.	1·7	19·1	1·18	14·53	0·582
2533	2	5.15 p.m.	3·5	16·7	1·12	12·12	0·572
2537	3	7 a.m.	7·2	13·2	1·04	8·92	0·569
2538	3	2 p.m.	14·0	11·3	0·95	—	0·558
2539	3	5.30 p.m.	15·2	11·1	0·87	7·93	0·557
2544	4	7 a.m.	4·1	9·3	0·92	4·10	0·549
2545	4	2 p.m.	5·3	9·1	0·84	—	0·560
2546	4	5.30 p.m.	4·3	9·0	0·87	4·12	0·541

* Determined in the standard Hortvet apparatus.

Schuette and Huebner (1933) confirmed the fact that the depression of colostrum is greater than that of the corresponding milk.

Effect on the freezing point of previous heating of milk

Hortvet (1921) refers to a paragraph in Leach (1920) (p. 143), where it is stated that Gooren affirms that homogenising, pasteurising and sterilising have the effect of lowering the freezing point; as regards pasteurising, Hortvet claims that this statement is wrong—the truth being that Gooren concluded that pasteurising sometimes changes the freezing point and sometimes does not. Monier-Williams found that sterilisation by heat causes a slight decrease in the freezing-point depression; Parker and Spackman (1929) found that the effect of pasteurisation was to cause a decrease of about 0·010°. Elsdon and Stubbs (1933b) examined fourteen milks when fresh and then after experimental pasteurisation and sterilisation in the laboratory. In some cases these processes had no effect on the freezing-point depression, whilst in the others there was a slight decrease. The maximum decrease noted for pasteurisation was 0·003° and for sterilisation 0·009° C.

These experiments suggest that the more ordinary processes of pasteurisation or sterilisation will not have such an effect on the freezing point of milk as to cause serious difficulty in interpreting the results obtained. The revisers are of the opinion that heating milk will have little if any effect on the freezing point, and that some of the alterations observed are due to difficulties of carrying out the necessary experiment without concentrating the milk by evaporation, or by diluting it by the steam used in the process. This opinion is supported by the fact that Elsdon and Stubbs (*loc. cit.*) heated a sample of milk in a closed container in an autoclave for 30 minutes at 110° C. The heated milk was quite brown in colour and had acquired a strong taste, but the freezing point was unchanged.

Williams (1936) examined milks which had been heated and kept hot under various conditions in cafés, buffets and snack bars. He found that the normal inference from the freezing point can be relied upon when a milk has been heated in any of the ways usual where hot milk is sold, except under some conditions when the milk has been reheated or allowed to evaporate to any extent, when added water may be somewhat under-estimated by this means.

The calculation of added water

Most of those who have considered the calculation of the amount of added water from the freezing points of mixtures of milk and water have adopted the method of simple proportion, as represented by the formula of the American Association of Official Agricultural Chemists:

$$W = \frac{100\,(T - T')}{T} \text{ per cent,}$$

where W is the amount of added water, T is the freezing-point depression of the original milk (or an average figure in cases where a comparison sample is not available), and T' the freezing-point depression of the sample.

There do not appear to be many recorded instances where a milk has been diluted, the freezing-point depression observed, and the amount of added water calculated from the freezing-point depression compared with the amount known to be present. Writers who have given some consideration to the matter (e.g. Monier-Williams) have thought that the above simple formula, whilst not being exactly correct, was yet sufficiently near the truth for practical purposes.

Hortvet (1921) published figures obtained from the examination of mixtures of milk with water, containing from 6 to 16 per cent of added water. He found that the amount of added water approximated closely to that present when calculated by the formula $W = \frac{100\,(T - T')}{T}$.

From a reading of *Methods of Analysis of the American A.O.A.C.* (7th edn., p. 238), there appears to be some ambiguity as to the method of expression used by the Association. It is, perhaps, natural to assume that the formula specified is intended to give percentages by weight, as it is similar in form to the corresponding formula $\frac{8{\cdot}5 - \text{s.n.f.}}{8{\cdot}5} \times 100$ used for calculation of added water from the solids-not-fat which, according to the instructions of the A.O.A.C., are determined by weight. There is, however, an alternative method indicated for the calculation of added water, viz. by means of Winter's table (Table 41.28 in the Appendix to *Methods of Analysis*), which gives the percentage *by volume*. When the added water is calculated by volume instead of by weight, the amount indicated will be higher. Twenty per cent by weight is equivalent to 20·5 per cent by volume.

Walder (1934) found that the calculated amount of water was usually greater than that actually present, but Plücker and Steinruck (1931) found that it was usually less (cf. also Buogo (1934)).

Elsdon and Stubbs (1936) found that a mixture of equal weights of water and of milk [F.P.D. (Hortvet) of milk 0·539° C] gave a freezing-point depression of 0·258° C. Calculating the amount of added water by the A.O.A.C. formula, we obtain 52·2 per cent, or 2·2 per cent above that actually present. In consequence of the result of this experiment, a series of determinations was carried out of freezing-point depressions of various mixtures of milk and water, with the following results—

Table 5.26—Freezing-point depressions of mixtures of milk and water
(*Elsdon and Stubbs*)

Actual added water per cent by weight	Total solids of mixture per cent by weight	F.P.D. found (Hortvet) °C	F.P.D. of original milk (Hortvet) °C	Water by A.O.A.C. formula per cent	Water by suggested formula (*infra*) per cent by weight
5	12·5	0·522	0·552	5·4	4·8
	12·1	0·516	0·547	5·7	5·0
10	11·4	0·484	0·547	11·5	10·2
	11·4	0·484	0·547	11·5	10·2
12½	10·7	0·470	0·546	13·9	12·5
	11·2	0·452	0·527	14·2	12·6
15	10·9	0·453	0·547	17·2	15·4
	10·6	0·452	0·546	17·2	15·4
	10·6	0·452	0·547	17·4	15·6
20	10·2	0·425	0·547	22·3	20·0
	10·2	0·424	0·547	22·5	20·2
25	9·6	0·399	0·547	27·1	24·5
	9·2	0·399	0·546	26·9	24·4
50	6·0	0·258	0·539	52·2	49·0
	6·4	0·261	0·547	52·3	48·9
75	3·2	0·133	0·547	75·7	73·3
90	1·3	0·060	0·547	89·1	87·9
	1·3	0·061	0·547	89·0	87·7

In order to attempt an explanation of these results and to endeavour to find a formula which will give results agreeing with the known composition of the mixtures, it is necessary to recall the laws governing the depression of the freezing points of dilute aqueous solutions.

For small differences in concentration of dilute solutions of non-ionised substances, the freezing-point depressions may be taken as proportional to the weight of substance dissolved in 100 g of the solvent. In the case of mixtures of milk and water, it is necessary to take into account several other factors, apart from this simple relationship. Thus it is necessary to consider—

(1) The effect of the weight of total milk-solids on the weight of solvent present.

(2) The dissociation of the electrolytes.

(3) Any departure from a straight line of the graph connecting concentration with freezing-point depression in the case of lactose, as occurs with cane sugar according to Raoult's formula,

$$\frac{18{\cdot}72 \times P}{342 - (0{\cdot}99 \times P)},$$

where P is the weight in grams of solute in 100 grams of solvent.

(4) The water of hydration of the lactose and any denaturation of the proteins (due to the splitting-off of water) during the drying of the total-solids, which will increase the apparent amount of " free " water present.

(5) The effect of any substances dissolved in the water used for the dilution of the milk, on the freezing point of the mixture; these will usually not exceed 1 per cent of the water added.

In the above formula the concentration of solutions is expressed as weight of solute in 100 g of solvent. The A.O.A.C. formula assumes that the same amount of solvent is present in the same quantity of all milks and mixtures of milk and water. This assumption is not correct and entails a considerable error. In milk of average quality, containing, say, 12·5 per cent by weight of total-solids, the active ingredients in 100 g of milk are dissolved in 100 — 12·5 = 87·5 g of water, whereas the A.O.A.C. formula assumes the solution to be in 100 g (or ml) of water. In the last column of the table are placed results corrected for the fact that the added water should be calculated by using weights of solvent and not of solution (that is, referring always to 100 g of water); and Elsdon and Stubbs suggested the revised formula—

$$\text{Added water} = \frac{T - T'}{T} \times (100 - T.S.) \text{ per cent by weight,}$$

where T is the freezing-point depression (Hortvet) of the original milk, T' the freezing-point depression (Hortvet) of the mixture, and $T.S.$ is the percentage of total-solids in the mixture.

In the above experiments T was determined. In practice T will not generally be known. Where comparison is being made with an appeal-to-cow sample the freezing-point depression of this will be used, but where no appeal-to-cow sample is available an average figure can be substituted.

The revised formula gives the amount of added water correctly (within the limits of experimental error) when this does not materially exceed 20 per cent. When the amount of added water is of the order of 25 per cent it is under-estimated by about 0·5 per cent. The under-estimation increases with the quantity of added water present, and becomes about 2 per cent when the added water is as high as 90 per cent. These results show a considerable improvement on the original formula, which invariably over-estimates added water where the amount does not exceed about 80 per cent. It was suggested by Elsdon and Stubbs that, in practice, the results given by the new formula can always be used, as under-estimation is not material in any instances which are likely to be met with in practice.

Of the five points mentioned above as possibly having some influence on the calculation of the amount of added water, the first, viz. the influence of the solids of the milk, is greatest when the amount of added water is least. It appears from the experimental results that the effect of the increase in dissociation of the electrolytes on dilution of the milk does not become evident until such dilution is at least 25 per cent. There is, however, the possibility—in fact, the likelihood—that some of the factors may work in opposite directions; if so, they will tend to cancel each other and thus permit of the use of a comparatively simple formula.

The third point mentioned above, that is, the possible association of the lactose, is not likely to be of serious moment. A 5 per cent solution of sucrose, i.e. 5 g of sugar in 100 g of water, has, according to Raoult's formula, a freezing-point depression of 0·2777° C, whilst a 3 per cent solution has a freezing-point depression of 0·1656° C; if the freezing-point depression of the 3 per cent solution were calculated from that of the 5 per cent by simple proportion, the figure would be 0·1666° C—a difference of only 0·001° C from the actual figure. Lactose is not unlikely to have a somewhat similar range.

The expression 100 — *T.S.* will not only include the "free" water in the milk (i.e. the water acting as a solvent for the crystalloids), but will also include any water of hydration which is attached to the lactose when in solution and which is expelled on drying, together with any water of combination of the proteins which is lost at the same time, i.e. more water than is present as solvent. The expression 100 — *T.S.* will, therefore, tend to be too high, and this will tend to give too high a figure for the amount of added water; this tendency may, however, be reduced (in high dilutions even reversed) by the effect due to dissociation of the electrolytes.

Elsdon and Walker suggested, therefore, that the results obtained from the formula

$$\text{Added water (per cent)} = \frac{T - T'}{T} \times 100$$

are too high, and that the following revised formula should be used—

$$\text{Added water (per cent wt./wt.)} = \frac{T - T'}{T} \times (100 - T.S.).$$

Davis (1951a) has suggested the formula—

$$\frac{0{\cdot}545 - T}{0{\cdot}545} \times (100 - 6{\cdot}8)$$

i.e. he considers 100 ml of milk as 93·2 ml of serum. This simplifies to

$$\text{Per cent added water} = 171\ D$$

where D = difference between observed depression and 0·545° C which is taken as the average depression.

(4) HYDROGEN ION CONCENTRATION

The pH is one of the most important factors controlling the behaviour of milk and dairy products.

The pH of milk

Milk freshly produced has a pH value usually varying between 6·5 and 6·7. Colostrum, or milk secreted immediately after the birth of the calf, is more acid, becoming almost normal after 3 and virtually normal in about 15 days. Milk of late lactation is more alkaline; and mastitis or disease of the udder almost invariably results in a more alkaline secretion. Kleckner (1940) has reported a sudden rise in the pH of milk during the period of oestrus.

Effect of heat on pH and buffer value of milk

Mild heating causes a loss of acidity or a slight rise in pH due to loss of CO_2 (Kirsten 1902, Whittier and Benton 1926). Continued heating at 100° C or higher results in a steady increase in acidity due to the formation of acids from the lactose and proteins (Orla-Jensen and Plattner 1905). The browning of milk, which results from humins formed by condensation of lactose degradation products with certain amino acids, is a reliable index of the degree of heating of milk at its ordinary pH value. If, however, it has been neutralised, the browning proceeds more easily as lactose is most stable slightly on the acid side of neutrality. Data on pH and titratable-acidity changes in heated milk have been given by Gould and Frantz (1945). Vodak and Tarassuk (1948) have shown that heating milk results in a lowering of buffer values, especially in the phosphate–citrate range. It is probable that this is due to the known heat-precipitation of tricalcium phosphate and, later, of tricalcium citrate. Cooling the milk results in partial recovery of the original buffer value. Chilling milk effects an increase in buffer value.

Methods of determining pH of milk

(*a*) *General*

Colorimetric methods are simplest for routine work where great accuracy is not required. The turbidity of milk necessitates a considerable dilution—usually about 1 in 20 to permit the use of a comparator. Sharp and McInerny (1926) have described a dilution method for milk, and Davis and Thiel (1940) have modified this for use with milk and whey in cheesemaking. Seekles (1940) found that " Lyphan " indicator strips agreed within 0·2 pH of electrometric methods but tended to give lower readings. An exhaustive paper by Døvle (1940) deals with factors influencing the accuracy of pH determinations in milk. He concludes that quinhydrone is satisfactory, using 2 g quinhydrone per litre of milk. Indicator strip paper methods gave low results. Schönberg (1942) has proposed nitrazine yellow as a pH indicator for milk. Fresh milk gives a blue-grey, souring milks a yellowish-blue and alkaline milks a strong blue. Ulrich (1944) has proposed a mixture of chlorphenol red and methylene blue as a substitute for litmus in milk.

(*b*) *Platform testing of farmers' supplies*

The need for a quick test on the creamery platform led Davis and his colleagues to explore the possibilities of a glass electrode for detecting souring milks. Although the technical difficulties were largely overcome, the high buffering power of milk renders the method unsatisfactory. Fresh normal herd bulks range in pH from 6·5 to 6·7. A rejection standard of less than disc 4 in the rapid or ten-minute resazurin test (Barkworth *et al.* 1943, Davis *et al.* 1943) is equivalent to a fall in pH of only about 0·2 units, which is the normal variation in initial pH values. It seems unlikely, therefore, that a simple pH test will ever be satisfactory for " weeding out " milks that have only just begun to sour. Further, mastitis and late lactation milks are more alkaline than normal milks, so that both pH and titratable-acidity measurements are unsatisfactory for platform testing *if a high standard is required.*

The alizarin–alcohol test is essentially the same, with the further disadvantage that the alcohol test will detect chemically unstable milks (occurring in April and May). However, for condensing this is an advantage, and the alizarin–alcohol test is possibly the best platform test for condensing creameries. Johnston (1944) found that there was no relation between pH and bacterial content, titratable acidity, chloride and leucocyte contents and resazurin value.

A point of interest to manufacturers of condensed and evaporated milk was that some relationship was observed between pH and protein stability.

Johnston and Doan (1943) have used an industrial pH meter under commercial conditions. A glass electrode and calomel half-cell were employed and a temperature correction applied. The frequency distribution of the results obtained is given in the following table—

Table 5.27—Frequency distribution of pH values of milk

pH value	*No. of results*
6·34	1
6·35–6·39	3
6·40–6·44	10
6·45–6·49	20
6·50–6·54	50
6·55–6·59	116
6·60–6·64	196
6·65–6·69	167
6·70–6·74	76
6·75–6·79	42
6·80–6·84	7
6·85–6·89	1
6·90–6·95	2

Since these workers did not determine *developed* acidity it is not possible to say to what extent low values were due to souring. The authors record, however, that milks with results lower than 6·40 have high plate counts. Milks with pH values of 6·8 or higher showed evidences of mastitis. Johnston and Doan suggest 6·4 to 6·8 as the limits for normal herd bulks, but a large majority of their milks fell in the range 6·55–6·7, and very few were outside the limits of 6·5–6·75. These workers did not observe any effect on pH due to season, although buffering power was slightly higher in spring and summer.

The average relationship between pH and titratable acidities of herd bulk milks has been found by Davis to be as follows—

Table 5.28

Titratable acidity per cent lactic acid	*pH*
0·14	6·60
0·19	6·20
0·24	5·94
0·34	5·60
0·44	5·30
0·54	5·04
0·64	4·80

Roeder (1948a) has published a critical treatment of the pH method for testing the freshness of milk. Grimmer and Arlart (1929) evolved the following formula relating pH to developed lactic acid—

$$y = c.x^2 \sqrt{k.S} = c.x^2 \sqrt{3{\cdot}375 \times 10^{-7}x}$$

where $k = 1{\cdot}35 \times 10^{-4}$ (dissociation constant of lactic acid),

$S = 2{\cdot}5 \times 10^{-3}x$ (lactic acid concentration in terms of titration by 0·25 N NaOH),

$\sqrt{k.S}$ or $\sqrt{3{\cdot}375 \times 10^{-7}x}$ = hydrogen ion concentration of a pure solution of lactic acid of titration x,

y = hydrogen ion concentration of milk,

and c = a buffer factor varying for milks from $3{\cdot}03$ to $5{\cdot}42 \times 10^{-6}$

Roeder transforms this equation on the basis of $\text{pH} = -\log y$ to

$$\text{pH} = 9{\cdot}236 - 2{\cdot}5 \log x - \log c$$

$$\text{or} \quad \log x = \frac{9{\cdot}236 - \text{pH} - \log c}{2{\cdot}5}$$

Roeder gives equivalent values for colorimetric, electrometric and titration methods for determining the acidity of various milks.

Methods for determining pH of cream

Wiley and Newman (1942) have published figures showing the relationship between the pH and titratable acidity of raw and neutralised cream and of buttermilk. Figures are also given for the pH of butter, buttermilk and the original cream. Townley and Gould (1941) have recorded that for neutralised cream the pH of the buttermilk is usually about 0·1 pH units less and that of the serum about 0·15 pH units more than that of cream.

Hankinson and Anderson (1941) have proposed a test for detecting neutralised cream based on a comparison of the pH and the buffering power of a uranyl nitrate serum. Lactic acid increases the buffering power and the neutraliser the pH. Parmelee *et al.* (1943) have compared the hydrogen, glass and quinhydrone electrodes for cream and butter. Appreciable differences were found between the electrodes, these being as high as 0·3 pH units for butter from lime-neutralised cream.

Methods for determining pH of butter

Butter may be made from fresh unripened (unsoured) cream or from ripened or soured cream. The latter has a much fuller flavour but is of poorer keeping quality, as acidity catalyses the oxidative deterioration by traces of metals. The pH of butter is most conveniently determined by separating the serum by warming the butter to 50° C and using the quinhydrone electrode on the serum (cf. B.S. No. 769—1938).

Loftus-Hills (1947) has shown that 10 per cent NaCl in milk reduces the pH by 0·42 units, and 0·2 per cent sodium citrate or phosphate by 1 unit. Differences in acidity between salted butter and cream used in its making may be due to many factors in the making process. Mohr and Baur (1940) found that sera from sour cream butter had pH values from 4·53 to 5·63 and sera from sweet cream butter values from 6·76 to 7·70. They studied the effect of storing butters at various temperatures and found no relation between deterioration in quality and change in pH. White (1944) records that creams of acidity

0·116, 0·203 and 0·31 per cent lactic acid produced butters of pH 6·65, 6·00 and 5·47. The acid butters were " metallic " in flavour when fresh, and tallowy after storage, although the copper content of all the butters was about the same. Virtanen (1947) has shown that oily and fishy flavours do not occur in butter if the serum pH is over 6. Keeping quality is improved by adding a mixture of Na_2CO_3 and Na_2HPO_4.

Methods for determining pH of cheese

Cheeses of the Cheddar type have a pH value of about 4·9 when in press, and during ripening this gradually rises to about 5·5 or higher (Davis 1932). Soft cheese may have an initial value lower than 4·9 and ultimately rise above 7 if the flora becomes more proteolytic. Mould-ripened cheeses similarly are initially strongly acid, but in the later stages of ripening the destruction of the lactic acid and production of ammonia by the moulds (*Penicillium*) may raise the pH above 7. Usually ripening proceeds faster at higher pH values, not only because the proteases are more effective but because the proteolytic types of bacteria grow better at less acid reactions.

The pH value of cheese is easily measured by direct mixing with a little water and quinhydrone. For routine work the mixture may be compressed into an open tube about 5 by 2 cm diameter, the gold electrode inserted into the paste and the column allowed to touch the saturated KCl in a beaker (Davis 1931) (cf. also B.S. No. 770—1938). Mulder (1946) has used the " Wulf pH tester " (indicator impregnated slip) for cheese and claims agreement with electrometric methods to within 0·1 pH units.

Dorner (1939) has emphasised the value of routine pH testing for quality control in Emmenthal cheese. At 24 hours the pH should be from 5·15 to 5·25. Virtanen (1941) finds that the pH of the curd must not be below 6·35 at the time of " dipping " the curd, and the pH of the cheese not above 5·3 at the end of pressing to get good cheese. After 2–3 months the pH rises to 5·5–5·7. Normal ripening does not take place below 5·5. Dolby *et al.* (1940) consider that pH determinations of Cheddar cheese at 14 days give a reliable indication of future quality, the best values being 4·90–4·94. Higher values were correlated with poorer grading. Later Dolby (1941a) emphasised that titratable acidities of whey are not necessarily an accurate measure of the pH of the curd. Divergences increase during the making process. The pH of whey at " whey off " exerts a considerable effect on the quality and subsequent behaviour of the cheese. Provided this is correct, earlier variations in acid development have little effect. " Dry stirring " of the curd resulted in a 2 per cent further loss of moisture and a high pH. Salting at too low a pH reduced the flavour and gave a harsh, mealy body.

pH of milk powder

Tarassuk and Richardson (1941b) have studied the reconstitution of milk powders and shown that whereas the use of pasteurised skim milk gave a reconstituted milk of fairly normal properties, the use of raw skim milk permitted lipolysis and so resulted in low pH and surface tension values. The reconstituted buttermilk did not clot with rennet. This phenomenon could be reproduced by adding steapsin to the original cream. The authors suggest that changes in the fat globule absorption membrane are responsible for this rapid lipolysis. Lowering of pH normally leads to more rapid rennet clotting and addition of caproic acid achieved this. However, lauric and the higher fatty acids inhibited coagulation. Warming at 44° C and quick cooling to 35° resulted in normal clotting.

pH of evaporated milk

This product is homogenised milk concentrated to about twice its original solids content and sterilised under pressure in tins. Its pH is, as might be expected, slightly less than that of fresh milk. Fortunately the heat stability of evaporated milk is at an optimum at pH 6·4–6·5. Stability decreases markedly over the range 6·4 down to 6·2 and is also less, rather curiously, above pH 6·5.

Condensed milk

Sweetened condensed milk is milk concentrated to about 2½ times its original solids content and contains about 38 per cent cane sugar. Skim condensed is skim milk concentrated to about twice its original solids and has about 44 per cent cane sugar present. The limits of pH do not seem to be as important as for evaporated milk.

pH of fermented or cultured milks

These may be grouped microbiologically into four classes—

(*a*) streptococci (up to 1 per cent lactic acid)
(*b*) low acid-forming lactobacilli (up to 1·5 per cent lactic acid)
(*c*) high acid-forming lactobacilli (over 1·5 per cent lactic acid)
(*d*) gas-forming yeast-inoculated types.

Group (*a*) attain a final pH of about 4·2, (*b*) about 3·8, (*c*) less than 3·8 and (*d*) about 5 but very variable (cf. Davis 1952b).

There is a close relationship between pH and developed lactic acid for the homofermentive cultures, but the relationship becomes erratic for the others and especially if yeasts are present.

pH of ice-cream

Very little attention has been paid to the pH aspects of ice-cream. In this material the final pH is controlled by that of the strongest buffering constituent. In a typical ice-cream mix the milk solids-not-fat component is by far the most important, the fat and sugar being negligible in this respect. The addition of acid flavours may reduce the pH slightly but normally it approximates to that of milk. Horrall *et al.* (1940) found that the pH values of commercial ice-cream varied from 5·62 to 7·05. Milk sherbets are naturally more acid on account of the added citric or tartaric acid.

Effect of pH on enzymes

All enzymes have characteristic pH ranges and this fact plays a very important part in the metabolism of living organisms. Milk is rich in some enzymes. Proteinases in milk have points of greatest activity at pH values of 5 and 7·5. Struble and Sharp (1940) found that rennet has a point of maximum stability st pH 4·0 at 48° C. Rapid destruction takes place at pH 3·5 and 4·5.

Milk lipase is most active at about pH 8·5, whereas bacterial lipases are most active at about pH 5.

Phosphatases have their characteristic pH optima and the milk phosphatase which forms the basis of the phosphatase test has an optimum at pH about 10 (Kay and Graham 1933); hence the incubation of the milk sample in the barbiturate buffer.

For a survey of the subject of pH in dairy products, see Davis (1950a).

Titratable acidity

Lines (1939) has drawn attention to the relationship between fat and titratable acidity and solids-not-fat. He also gives figures showing the effect of breed, stage of lactation, season and milking time. Døvle (1949) has failed to find any relationship between titratable acidities and pH values in milk. Robinson and Samson (1946) give the following results for high acidity milks from Jersey cows—

		Correlation coefficient against acidity
Titratable acidity	0·1717	
Free CO_2, vol. per cent	6·499	– 0·38
Fixed CO_2, vol. per cent	0·701	0·086
Citric acid, mg per cent	42·38	– 0·47
Casein per cent	2·838	0·355
Albumin and globulin, per cent	0·576	0·216
Inorganic phosphorus, mg per cent	63·45	0·738

(5) OTHER PHYSICAL CHARACTERISTICS

Surface tension and surface activity

Aschaffenburg (1946) has shown that the effect of dilution on the surface tension of milk throws an interesting light on the substances responsible for surface tension effects. The most important appears to be " proteose " in nature, and, although heterogeneous, consists of one component to the extent of about one-half. Casein is also important but not the albumin and globulin. Fat reduces the surface tension, probably by a physical effect. Whitnah *et al.* (1949) have used the vibrating jet method to show that the surface tension of fresh surfaces of homogenised milk falls rapidly from a value which approaches that of water. With diluted milks the fall can be followed over a period of hours.

Foaming

El Rafey and Richardson (1944) have studied the ability of milk constituents to produce foam. In synthetic solutions lactalbumin, calcium caseinate, triglycerides and phospholipids can produce foams. Below 27° C caseinate is preferentially absorbed at the skim milk–air interface; at higher temperatures lactalbumin replaces casein. Fat is responsible for the low foaming powers at 27° C and protein–phospholipid complexes for the foaming of cream. Quite small amounts of fat seriously reduce foam stability. This effect is more apparent above the melting point of fat for casein and below it for albumin.

The foam stability of separated milk and whey can be markedly increased by repeated super-centrifuging, and fat-free milk gives stable foams over a wide temperature range, the zone of minimum foaming (20°–30° C) disappearing. The importance of casein as a foam promoter is illustrated by the fact that separated milk forms a more stable foam than whey. There appears to be no relation between foam stability and the surface activity of the protein system. In a further paper (Richardson and El Rafey 1948) they point out that foaming is at a minimum at 30°–35° C and that at 60° C the foam volume is independent of the fat content.

Fluorescence

Jenness and Coulter (1948) have fractionated the fluorescent substances in milk as follows—

(1) Soluble in 67 per cent acetone ..	Riboflavin (and proteins, etc.).
(2) Soluble in acetone–ether (20 : 80) ..	Phospholipides (and other lipides).
(3) Soluble in 10 per cent KCl	Proteins.

Colour

Nelson (1948) has used a Beckman spectrophotometer to measure the colour of milk. Evaporated milk has been studied using reflectance at 520 mμ. Choi *et al.* (1949b) have overcome the difficulty caused by absorption of colouring matter on proteins by tryptic hydrolysis. They measure photometrically the colour of a trichloracetic acid filtrate of the hydrolysed mixture.

Viscosity

Bogdanov (1940) has shown that Einstein's law does not hold for products such as koumiss and has obtained empirically an equation very similar to that of Hatschek—

$$\eta = \frac{1}{1-\sqrt[3]{\phi}}$$

where η = viscosity and ϕ = ratio of protein volume to total volume.

Potter *et al.* (1949) have emphasised the advisability of employing the same standardised instrument throughout a series of tests. Variation in technique caused a change in the apparent viscosity. Spöttel and Gneist (1942) have found that the higher viscosity of evening milk cannot be accounted for by the increased fat content. Viscosity appeared to be correlated with s.n.f. and with total solids but not with fat, protein and fat + protein.

In his studies of the viscosity–temperature coefficient of homogenised milk Caffyn (1951) finds that the viscosity falls by about 2×10^{-4} poise at 80° C. A slight rise in viscosity curves at temperatures about 60° C is probably due to changes in the protein structure of the milk.

Vapour pressure

Scott-Blair *et al.* (1941) have measured the vapour pressure of cheese by sealing in tins with salt solutions of known vapour pressure. They suggest that similar methods may be of use for watered milks.

CHAPTER 6

FORMULAE FOR MILK CALCULATIONS

The specific gravity of milk is raised by the solids-not-fat and lowered by the fat. This fact is not only true qualitatively but also, as the following demonstration will show, quantitatively.

By the definition that specific gravity (S) is the weight (W) of unit volume (V), we get the equation—

$$S = \frac{W}{V}. \qquad . \quad . \quad . \quad . \quad . \tag{1}$$

Let us suppose we have a mixture (having specific gravity S) of two substances, A and B, of differing specific gravities S_A and S_B. Suppose further that the respective weights are A and B, and let $A + B = 100$.

Then by (1),

Volume of $A = \frac{A}{S_A}$, volume of $B = \frac{B}{S_B}$, and volume of mixture $= \frac{100}{S}$.

Then

$$\frac{100}{S} = \frac{A}{S_A} + \frac{B}{S_B} = \frac{A}{S_A} + \frac{100-A}{S_B}$$

$$= \frac{A(S_B - S_A)}{S_A S_B} + \frac{100}{S_B}.$$

$$\frac{100}{S} - \frac{100}{S_B} = A\left(\frac{S_B - S_A}{S_A S_B}\right)$$

$$1 - \frac{S}{S_B} = AS\left(\frac{S_B - S_A}{100 S_A S_B}\right)$$

$$S = S_B + AS\left(\frac{S_A - S_B}{100 S_A}\right).$$

Now in the same way,

$$S = S_A + AS\left(\frac{S_B - S_A}{100 S_B}\right).$$

As both S_A and S_B are constants, we may write—

$$\left.\begin{aligned} S &= S_A + BS \times K_B \\ \text{and} \quad S &= S_B + AS \times K_A \end{aligned}\right\} K_B \text{ and } K_A \text{ being constants.} \quad . \quad . \tag{2}$$

Now, as $A + B = 100$, A is the percentage by weight of this substance; and as $100 \times S$ expresses the total number of grams in 100 ml of the mixture, AS is the number of grams of this substance in 100 ml.

From the equations given above we can deduce the law that " If two substances of differing specific gravity be mixed, the specific gravity of the mixture will be equal to the specific gravity of one of the substances *plus* the number of grams of the other per 100 ml of the mixture multiplied by a constant factor ".

Regarding milk as a mixture of fat and a solution of solids-not-fat in water, we can say that the specific gravity of a milk is equal to the specific gravity of the solution of solids-not-fat *plus* the number of grams of fat per 100 ml multiplied by a constant.

In the *solution* of solids-not-fat we have, in 100 ml, x grams of solids-not-fat; let us assume that their density is y. Then x grams will occupy a volume $\frac{x}{y}$. Let the specific gravity of the solution be S. The 100 ml weigh $100\ S$ grams, and the water in this weighs $100\ S - x$ grams; it also measures $100 - \frac{x}{y}$ ml. Now, as the specific gravity of water is 1,

$$100\ S - x = 100 - \frac{x}{y}$$

$$100\ S = 100 + x - \frac{x}{y}$$

$$S = 1 + x\left(\frac{y-1}{100\ y}\right) \quad . \quad . \quad . \quad . \quad (3)$$

Now, $\frac{y-1}{100\ y}$ is a constant, provided that y remains constant. Putting the equation into words, we find that " The specific gravity of a solution of solids-not-fat is equal to 1 *plus* the number of grams of solids-not fat in 100 ml multiplied by a constant ". It is known, however, that the specific gravity of substances in solution is not quite constant, but varies slightly with dilution.

The following figures (Table 6.1) will show that in milk the law just enumerated holds good within the limits of experimental error. A poor skim-milk was diluted with water, and the total solids and specific gravity at 15·55° estimated—

Table 6.1—Relation between solids and specific gravity of skim-milk

Total solids per cent	Specific gravity	Constant
9·280	1·03544	0·003688
8·758	1·03343	0·003693
8·318	1·03170	0·003694
7·777	1·02950	0·003684
7·456	1·02829	0·003690
6·455	1·02439	0·003688

From the laws expressed by equations (2) and (3) we see that the specific gravity of an aqueous liquid containing a substance in solution or in admixture can be expressed equally well as a direct multiple of the number of grams per

100 ml; for if we suppose that the substance A is water, S_A will equal 1, and equation (2) can then be written—

$$S = 1 + BS \times K_B,$$

which is practically equation (3).

In order to deduce a formula expressing the relation between specific gravity and percentage by weight of fat and solids-not-fat, let us call the specific gravity (for convenience) $1 + S$, the percentage by weight of fat F, and of solids-not-fat N.

Then the number of grams of fat per 100 ml will be $F \times (1 + S)$ and of solids-not-fat $N \times (1 + S)$.

The weight of the water in 100 ml is then

$$100 \times (1 + S) - N \times (1 + S) - F \times (1 + S) \text{ grams}$$

and its volume

$$100 \times (1 + S) - N \times (1 + S) - F \times (1 + S) \text{ ml}$$

The volume of fat and solids-not-fat in 100 ml is therefore

$$100 - [100 \times (1 + S) - N \times (1 + S) - F \times (1 + S)] \text{ ml},$$

which equals

$$N \times (1 + S) + F \times (1 + S) - 100\,S. \quad . \quad . \quad . \quad (4)$$

Let us assume that the specific gravity of fat is f and of solids-not-fat n.

Then the volume of fat in 100 ml is $\frac{F \times (1 + S)}{f}$ and of solids-not-fat $\frac{N \times (1 + S)}{n}$; therefore

$$N \times (1 + S) + F \times (1 + S) - 100\,S = \frac{F \times (1 + S)}{f} + \frac{N \times (1 + S)}{n},$$

or

$$100\,S = N \times (1 + S) - \frac{N \times (1 + S)}{n} + F \times (1 + S) - \frac{F \times (1 + S)}{f},$$

or

$$100\,S = N \times (1 + S)\left(\frac{n-1}{n}\right) + F \times (1 + S)\left(\frac{f-1}{f}\right).$$

Now, as n and f are constant, we may write for $\left(\frac{n-1}{n}\right)$, a; and for $\left(\frac{f-1}{f}\right)$, b.

Then the equation stands—

$$\frac{100\,S}{1 + S} = aN + bF. \quad . \quad . \quad . \quad . \quad (5)$$

It is usual, however, to estimate total solids (T) and fat in an analysis.

$T = N + F$, and therefore $N = T - F$.

The equation (5) may be written—

$$\frac{100\,S}{1 + S} = a\,(T - F) + bF,$$

or

$$\frac{100\,S}{1 + S} = aT + (b - a)\,F. \quad . \quad . \quad . \quad (6)$$

The specific gravity of milk is usually expressed in lactometer degrees, which are the specific gravity multiplied by 1,000 *minus* 1,000.

Thus if the specific gravity be 1·032, the lactometer degrees are 1·032 × 1,000—1,000 = 32.

Let us express lactometer degrees by the symbol G.

Then $G = 1{,}000\ S$, and, substituting this in (6), we get

$$\frac{G}{1 + S} = 10aT + 10\,(b - a)\,F.$$

The specific gravity was expressed as $1 + S$ for each calculation; it is better, however, to substitute the symbol D in the formula, which then stands as

$$\frac{G}{D} = 10aT + 10\,(b - a)\,F,$$

or

$$T = \frac{1}{10a} \times \frac{G}{D} - \left(\frac{b - a}{a}\right) F. \qquad (7)$$

As, by the definition above, $a = \frac{n - 1}{n}$ and $b = \frac{f - 1}{f}$ we could calculate a formula if we knew the specific gravities of solids-not-fat and fat, but we do not know both of these. Fleischmann has determined the specific gravity of the fat of milk to be 0·9307 at 15° C/15° C, but it is impossible to determine the specific gravity of solids-not-fat in solution. Moreover, Fleischmann's determination of the specific gravity was made on fat in the solid state, and it is possible that in milk it may have a different specific gravity.

By transforming equation (7) into the form

$$1 = \frac{1}{10a} \times \frac{G}{TD} - \left(\frac{b - a}{a}\right) \times \frac{F}{T}$$

and making a large number of determinations of $\frac{G}{D}$, T, and F in different milks, we can form each pair of results into simultaneous equations and solve them. In this way we can get a large number of values for a and b, and, from the mean of these, we can calculate the specific gravities of fat and solids-not-fat respectively. This method is not wholly free from objection, because unless there is a considerable difference between the figures actually determined, the figures from which a and b are calculated are very small and are therefore affected greatly by experimental error; while if the difference be large (as in the case of analyses of cream and skim-milk), it is found that the experimental error is also increased. For this reason, and also for the reason that the specific gravities of fat and solids-not-fat are themselves liable to slight variations, it is necessary to deduce the formula from a great many determinations, thereby involving much labour in calculating.

In order to ascertain the specific gravities of fat and solids-not-fat in milk, Richmond calculated their value from over 200 analyses made by the most exact methods at his command, and found the following figures—

Specific gravity of fat	0·93
,, ,, solids-not-fat	1·616

Leonard has calculated by the method of least squares from a large series of results the factors 0·931 and 1·613, which are practically identical with the above.

It is seen that Richmond's figure, as well as Leonard's, calculated from actual analyses, agree with Fleischmann's determination of the specific gravity of fat.

As the specific gravity of milk does not vary much, it will not make an appreciable error if, instead of $\frac{G}{D}$, the expression $\frac{G}{1{\cdot}0302}$ be used; this form of calculation is much easier.

The idea of deducing a relation between specific gravity, fat, and total solids appears to have arisen with Behrend and Morgen, who published a table.

Fleischmann and Morgen (1885, 1886) next published a formula in which the specific gravity of fat was assumed to be 0·94; this was corrected by Fleischmann after his determination of the specific gravity of fat as 0·93. His formula is

$$T = 0{\cdot}2665\,\frac{G}{D} + 1{\cdot}2\ \mathrm{F}.$$

Hehner and Richmond deduced the formula[1]—

$$T = 0{\cdot}254\,G + 1{\cdot}164\,F.$$

This is in the less scientifically correct, but more convenient form; as it was found that milk differing appreciably in specific gravity from 1·0320 did not give results which agreed well with the formula, various approximations have been made to this.

Richmond calculated another formula which gives practically the same results but is more scientifically correct, and which does not require the application of approximations. This is—

$$T = 0{\cdot}262\,\frac{G}{D} + 1{\cdot}17\,F.$$

As the previous formulae were calculated from analyses to which objection could be taken, Richmond deduced a new formula from the results of analyses made as exactly as possible, viz.—

$$T = 0{\cdot}2625\,\frac{G}{D} + 1{\cdot}2\,F.$$

This has been found to be expressed by the simpler formula—

$T = \frac{G}{4} + \frac{6}{5}F + 0{\cdot}14$, within very small limits, if the specific gravity lies between 1·020 and 1·036.

Fleischmann has given the approximation formula—

$T = \frac{G}{4} + 1{\cdot}2\,F + 0{\cdot}25$; the formula s.n.f. $= \frac{G+F}{4}$ gives a fair approximation with average milks.

The formula $T = \frac{G}{4} + \frac{6}{5}F$ also approximates closely to that of Hehner and Richmond.

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[1] In the original paper a slight correction for skim-milks was included in the formula; this has now been abandoned.

Other formulae were derived by Brown, Babcock, Leonard, Lythgoe and others. Later investigators in this field include Bouriez (1921), Perkins (1931), de Waal (1932), Hawley (1933a) and Cini (1933).

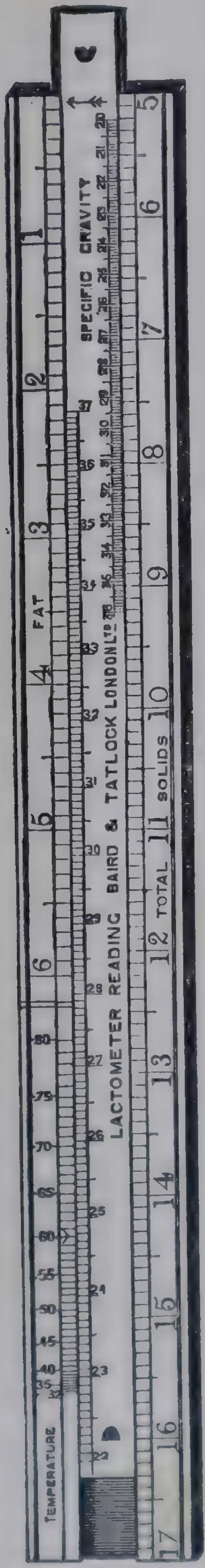

Fig. 1
Milk scale

Milk scale

In order to save calculation, Richmond devised a slide rule, known as the " milk scale " (Fig. 1), from which the percentage of fat can be read off directly from the specific gravity and percentage of total solids. On one side a scale is placed indicating total solids, 1 per cent of total solids being represented by 1 inch; on the other side the fat is shown by a scale of 1·2 ($1\frac{1}{5}$ inches) to 1 per cent; the slide carries the specific gravity scale, 1° being equal to 0·25 ($\frac{1}{4}$) inch. The line indicating the specific gravity found is placed against the total solids determined; an arrow placed 0·14 ($\frac{1}{7}$) inch from the end of the specific gravity scale then gives the fat as calculated by the formula

$$T = 0{\cdot}25\,G + 1{\cdot}2\,F + 0{\cdot}14.$$

To facilitate reading, Cassal and Gerrans (1902) suggested adding two sliding pointers, one on the total solids scale, and one on the specific gravity slide, which were first placed against the part of the scales corresponding to total solids and specific gravity found respectively, and the two pointers then adjusted. This arrangement, it was claimed, prevents the possibility of error in adjusting the slide.

Richmond also employed a runner made from a piece of brass bent round the milk scale, in which two holes are cut, leaving a narrow bar between them; this bar partially covers both the total solids and specific gravity scales, and has a fine line drawn upon it at right angles to the scales. By adjusting this line to the total solids, and the specific gravity to the line, the object sought by Cassals and Gerrans is attained. Stokes used a strip of transparent celluloid on which a fine line was drawn. The idea of the runner is due to Lieut. Mannheim of the French Artillery, who in 1851 devised it for a logarithmic slide rule.

The scales are divided into tenths, hundredths being estimated by the eye; a decimal scale, or better a vernier, as suggested by Sykes, can be applied to the runner, rendering it easier to read the second place of decimals. This, however, is not necessary, the error due to unavoidable circumstances being greater than the error of computation of the hundredths.

The total solids calculated on Richmond's scale from the specific gravity and the fat determination usually agree within 0·1 per cent, and almost invariably within 0·2 per cent, with a determination made by the appropriate gravimetric method.

Approximate figures for solids-not-fat and fat respectively can also be calculated from Tables 6.2 and 6.3; these will be found sufficiently accurate for routine determinations.

Table 6.2—For calculating solids-not-fat from fat and specific gravity

Correction	* Specific gravity (degrees)							
−1·0	25·0	25·5	26·0	26·5	27·0	27·5	28·0	28·5
*	**29·0**	**29·5**	**30·0**	**30·5**	**31·0**	**31·5**	**32·0**	**32·5**
+1·0	33·0	33·5	34·0	34·5	35·0	35·5	36·0	36·5
Fat	Solids-not-fat							
0·0	7·40	7·50	7·65	7·75	7·90	8·00	8·15	8·25
0·25	7·45	7·55	7·70	7·80	7·95	8·05	8·20	8·30
0·5	7·50	7·60	7·75	7·85	8·00	8·10	8·25	8·35
0·75	7·55	7·65	7·80	7·90	8·05	8·15	8·30	8·40
1·0	7·60	7·70	7·85	7·95	8·10	8·20	8·35	8·45
1·25	7·65	7·75	7·90	8·00	8·15	8·25	8·40	8·50
1·5	7·70	7·80	7·95	8·05	8·20	8·30	8·45	8·55
1·75	7·75	7·85	8·00	8·10	8·25	8·35	8·50	8·60
2·0	7·80	7·90	8·05	8·15	8·30	8·40	8·55	8·65
2·25	7·85	7·95	8·10	8·20	8·35	8·45	8·60	8·70
2·5	7·90	8·00	8·15	8·25	8·40	8·50	8·65	8·75
2·75	7·95	8·05	8·20	8·30	8·45	8·55	8·70	8·80
3·0	8·00	8·10	8·25	8·35	8·50	8·60	8·75	8·85
3·25	8·05	8·15	8·30	8·40	8·55	8·65	8·80	8·90
3·5	8·10	8·20	8·35	8·45	8·60	8·70	8·85	8·95
3·75	8·15	8·25	8·40	8·50	8·65	8·75	8·90	9·00
4·0	8·20	8·30	8·45	8·55	8·70	8·80	8·95	9·05
4·25	8·25	8·35	8·50	8·60	8·75	8·85	9·00	9·10
4·5	8·30	8·40	8·55	8·65	8·80	8·90	9·05	9·15
4·75	8·35	8·45	8·60	8·70	8·85	8·95	9·10	9·20
5·0	8·40	8·50	8·65	8·75	8·90	9·00	9·15	9·25
† Change at	0·2	0·1	0·2	0·1	0·2	0·1	0·2	0·1

* The solids-not-fat are correct for the middle line of specific gravity; if the specific gravity falls in the top line, subtract 1 from the solids-not-fat; thus 26·0 specific gravity and 3·0 per cent fat give 7·25 per cent solids-not-fat; if the specific gravity falls in the bottom line, add 1 to the solids-not-fat; thus 34·0 specific gravity and 3·0 per cent fat give 9·25 per cent solids-not-fat.

† The last line indicates where the change of solids-not-fat takes place; in a column with 0·2 at the bottom, use the column itself for the percentage of fat given, and 0·05, 0·10, and 0·15 above, but use the figure immediately below for 0·2 above; thus 30·0 specific gravity and 3·1 per cent fat give 8·25 per cent solids-not-fat, but 30·0 specific gravity and 3·2 per cent fat give 8·30 per cent solids-not-fat; in a column with 0·1 at the bottom, use the column itself for the percentage of fat given and 0·05 above, but change to the figure immediately below for 0·1 or more above; thus 30·5 specific gravity and 3·30 per cent fat give 8·40 per cent solids-not-fat, but 30·5 specific gravity and 3·40 per cent fat give 8·45 per cent solids-not-fat.

Table 6.3—For calculating fat from total solids and specific gravity

Specific gravity observed										
↓	A25·0 B29·0 C33·0	25·5 29·5 33·5	26·0 30·0 34·0	26·5 30·5 34·5	↓	A27·0 B31·0 C35·0	27·5 31·5 35·5	28·0 32·0 36·0	28·5 32·5 36·5	C ↓
Total solids A	Fat				Total solids B	Fat				Total solids C
7·00	0·50	0·40	0·30	0·20	8·00	0·10	—	—	—	9·00
7·25	0·70	0·60	0·50	0·40	8·25	0·30	0·20	0·10	—	9·25
7·50	0·90	0·80	0·70	0·60	8·50	0·50	0·40	0·30	0·20	9·50
7·75	1·15	1·05	0·90	0·80	8·75	0·70	0·60	0·50	0·40	9·75
8·00	1·35	1·25	1·15	1·05	9·00	0·90	0·80	0·70	0·60	10·00
8·25	1·55	1·45	1·35	1·25	9·25	1·15	1·05	0·90	0·80	10·25
8·50	1·75	1·65	1·55	1·45	9·50	1·35	1·25	1·15	1·05	10·50
8·75	1·95	1·85	1·75	1·65	9·75	1·55	1·45	1·35	1·25	10·75
9·00	2·15	2·05	1·95	1·85	10·00	1·75	1·65	1·55	1·45	11·00
9·25	2·40	2·30	2·15	2·05	10·25	1·95	1·85	1·75	1·65	11·25
9·50	2·60	2·50	2·40	2·30	10·50	2·15	2·05	1·95	1·85	11·50
9·75	2·80	2·70	2·60	2·50	10·75	2·40	2·30	2·15	2·05	11·75
10·00	3·00	2·90	2·80	2·70	11·00	2·60	2·50	2·40	2·30	12·00
10·25	3·20	3·10	3·00	2·90	11·25	2·80	2·70	2·60	2·50	12·25
10·50	3·40	3·30	3·20	3·10	11·50	3·00	2·90	2·80	2·70	12·50
10·75	3·65	3·55	3·40	3·30	11·75	3·20	3·10	3·00	2·90	12·75
11·00	3·85	3·75	3·65	3·55	12·00	3·40	3·30	3·20	3·10	13·00
11·25	4·05	3·95	3·85	3·75	12·25	3·65	3·55	3·40	3·30	13·25
11·50	4·25	4·15	4·05	3·95	12·50	3·85	3·75	3·65	3·55	13·50
11·75	4·45	4·35	4·25	4·15	12·75	4·05	3·95	3·85	3·75	13·75
12·00	4·65	4·55	4·45	4·35	13·00	4·25	4·15	4·05	3·95	14·00
12·25	4·90	4·80	4·65	4·55	13·25	4·45	4·35	4·25	4·15	14·25
12·50	5·10	5·00	4·90	4·80	13·50	4·65	4·55	4·45	4·35	14·50
12·75	5·30	5·20	5·10	5·00	13·75	4·90	4·80	4·65	4·55	14·75
13·00	5·50	5·40	5·30	5·20	14·00	5·10	5·00	4·90	4·80	15·00

If the total solids do not exactly agree with a figure in the Table, add the excess of total solids to the fat; thus, given 30·5 specific gravity and 11·8 per cent total solids add 0·05 to fat corresponding with 11·75 per cent total solids = 3·35 per cent fat.

If the specific gravity lies in line marked A, use the total solids column marked A; if in B use total solids column B; if in C use total solids column C.

Density calculations

Richmond's formula has been adapted to calculations involving the determination of density by the use of density hydrometers. Complete series of tables for use with these hydrometers are given in B.S. No. 734—1937[1], and as indicated on p. 319, a modified " milk scale " is also obtainable.

Specific gravity of constituents

By our definitions (p. 158),

$$a = \frac{n-1}{n} \quad \text{and} \quad b = \frac{f-1}{f},$$

and from the values given above in the formulae mentioned, *a* and *b* can be calculated.

Thus, taking the formula $T = 0{\cdot}2625\,\frac{G}{D} + 1{\cdot}2\,\mathrm{F}$,

$$0{\cdot}2625 = \frac{1}{10a} \text{ and } 1{\cdot}2 = -\frac{b-a}{a} \text{ or } \frac{a-b}{a}$$

therefore $a = 0{\cdot}381$ and $b = -0{\cdot}0762$

now $n = \frac{1}{1-a}$ and $f = \frac{1}{1-b}$

and therefore $n = 1{\cdot}616$ specific gravity of solids-not-fat

and $f = 0{\cdot}930$,, ,, fat

From the mode of deducing equation (7) (p. 158) we see that *a* is the number of grams that the weight of 100 ml of milk is greater than the weight of 100 ml of water, when 1 gram per 100 ml of solids-not-fat is contained therein, the specific gravity being, by definition, the weight of 1 ml. $\frac{a}{100}$ and $\frac{b}{100}$ are respectively the differences in specific gravity due to 1 gram per 100 ml of solids-not-fat and of fat.

It is also apparent that we may calculate from any analysis the amount that 1 gram of total solids per 100 ml has raised the specific gravity, and, from this, the specific gravity of the total solids.

Thus, using the same symbols as before,

$$T = \frac{G}{D} \times \frac{1}{10x} \quad \text{and} \quad t = \frac{1}{1-x}$$

(*t* here representing the specific gravity of the total solids).

Thus, if a milk has a specific gravity of 1·032 and contains 12 per cent of total solids,

$$12 = \frac{32}{1{\cdot}032} \times \frac{1}{10x} \quad \text{and} \quad x = 0{\cdot}2584$$

and $t = 1{\cdot}348$

It is occasionally useful to calculate the specific gravity of the total solids of a milk, as the total solids of skimmed milk have a considerably higher specific gravity than those of whole milk.

Instead of taking the solids-not-fat as one substance, we may consider its constituents—lactose, protein, and mineral matter—separately.

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[1] Published by the British Standards Institution, 28 Victoria Street, London, S.W.1.

Calling the percentage of fat F, of lactose L, of protein P, and of mineral matter A, we may write—

$$\frac{G}{D} = 10bF + 10cL + 10dP + 10eA$$

by the same reasoning employed in deducing formula (5) (p. 157).

Richmond has deduced from the mean of many analyses the formula

$$\frac{G}{D} = -0\cdot761\ F + 4\ L + 2\cdot5714\ P + 8\cdot46\ A$$

From these factors the following specific gravities are deduced, as previously shown—

Specific gravity of fat	0·93
,, ,, lactose	1·666
,, ,, protein	1·346
,, ,, mineral matter	5·5

Using these specific gravities, together with Vieth's estimate of the proportions of lactose, protein, and mineral matter—13 : 9 : 2—we can calculate what the specific gravity of solids-not-fat should be. As Vieth's proportions are by weight, we must transform specific gravities into specific volumes.

Specific volume of lactose $= \dfrac{1}{1\cdot666} = 0\cdot6$

,, ,, protein $= \dfrac{1}{1\cdot346} = 0\cdot743$

,, ,, mineral matter $= \dfrac{1}{5\cdot5} = 0\cdot182$

Then specific gravity of s.n.f.

$$= \frac{1}{\dfrac{13 \times 0\cdot6 + 9 \times 0\cdot743 + 2 \times 0\cdot182}{24}} = \frac{1}{0\cdot6188} = 1\cdot616,$$

which agrees with that given above (p. 158).

It is a useful check on an analysis to calculate the specific gravity from the percentage of milk-sugar, protein, fat, and mineral matter; this should agree within small limits with that found.

CHAPTER 7

DETECTION OF THE ADULTERATION OF MILK

(1) ADDED WATER

It would be useless to affirm that the problem of the detection of added water in milk is an easy one, since it has taken some seventy years of evolution and modification of methods to provide the answer. How far this problem has even now been solved will be seen by reference to the outline of the subject which is given below; it can at least be said that analysts are in a better position to-day to give an opinion than they were for many years subsequent to the passing of the Food and Drugs Acts of 1875.

The total constituents of milk, apart from the water, are, as we have seen, classed together as " total solids ", and these amount, on the average, to 12·65 per cent. Of this 12·65 per cent solid matter, 3·75 per cent is fat, the remainder being described as " solids-not-fat " and amounting, again on the average, to 8·9 per cent of the milk. If, therefore, we determine the percentage of fat and solids-not-fat in an average milk, we shall obtain the figures 3·75 and 8·90 respectively. If then we take equal volumes of water and milk and mix them, the percentages of fat and of solids-not-fat in the mixture will be half those in the original milk, because the amount of the ingredients which were present in 100 parts of the milk will now be present in 200 parts of the mixture. A similar proportionality will be shown by other mixtures, as given in the following table—

Table 7.1—Composition of average milk mixtures with water

Percentage of milk	Solids-not-fat	Fat	Water
0	0·00	0·00	100·00
25	2·22	0·93	96·85
50	4·45	1·88	93·57
75	6·68	2·82	90·50
95	8·45	3·56	87·99
100	8·90	3·75	87·35

The sample containing 50 per cent of *added* water contains 93·57 per cent of water in the mixture, and it is not unusual to find a certain confusion in the minds of some that it is possible to add 50 per cent of water to a liquid which already contains 87·35 per cent and form a mixture which contains only 93·57 per cent. When the question is put in this way there is apparently a little difficulty, but the explanation is easily arrived at. The parts of total solids represent 100 parts of milk. If we add 10 per cent of water to the milk, we shall reduce the amount of total solids by 10 per cent, that is, we reduce 12·65 parts by 10 per cent. Now 10 per cent of 12·65 is 1·27, so that by adding 10 per cent of water to an average milk, we reduce the total solids by 1·27 per cent, i.e. from 12·65 to 11·38 per cent. In an average milk the total solids are 12·65 per cent, and the water is therefore 87·35 per cent; in an average milk to which 10 per cent of water has been added these figures are 11·38 and 88·62 respectively. From this it will be seen that the actual amount of water present is increased only from 87·35 to 88·62 when 10 per cent of water is added.

Up to this point we have been considering average milks. Now milk, being a natural secretion, is subject to variation in its composition (as are all such natural products) both from animal to animal and from herd to herd. The conception of an average milk is quite legitimate from the mathematical point of view, but in practice it is seldom realised. The milks from different herds vary somewhat widely in total solids, figures from less than 10 to more than 15 per cent having been reported, although such wide variations are most unusual, the figures obtained lying between 11·5 and 13·0 per cent in all but a few cases. It has been found, however, that the fat varies much more than the solids-not-fat, which latter figure usually lies within the limits 8·6 and 9·1, although occasionally smaller and larger percentages are found. For this reason it has been customary for a considerable number of years to use the percentage of solids-not-fat as a basis for calculating added water.

The figures contained in the milk regulations (see p. 304) were not intended, either literally or by implication, to be taken as standards for milk. A milk which contains less than 8·5 per cent of solids-not-fat is not necessarily adulterated, and one that contains 8·5 per cent or more is not necessarily genuine.

All that the regulations do is to move the onus of proof. In the case of the prosecution of a vendor of milk for a sample which contained 8·5 per cent or more of solids-not-fat, the Local Authority would have to prove that the sample was adulterated; in the case of a prosecution for a sample which contained less than 8·5 per cent of solids-not-fat, the defendant, in order to escape conviction, would have to prove the milk to be genuine.

The weight which has been given to this limit of 8·5 per cent of solids-not-fat has varied considerably. There are those who appear to consider that it is almost an absolute minimum, and that any milk which contains less than this amount is almost certainly watered; others, however, attach little importance to this figure.

In the years 1930 and 1931, 5,959 samples of milk taken under the Food and Drugs Act were examined in the Lancashire County Laboratory. Of this number 121, or 2·0 per cent, contained less than 8·5 per cent of solids-not-fat. By means of some of the methods described below, each of these deficient samples was examined for the presence of added water, and it was found that 102 contained added water, whilst 19 were naturally poor. It follows, then, as far as these samples are concerned, that in the case of herds of cows, only 0·3 per cent give milk containing less than 8·5 per cent of solids-not-fat. From this it must of necessity follow that the limit of 8·5 is at least a very good sorting test. It is, in fact, far more likely to fail to detect slightly adulterated milks (containing, say, from 1 to 5 per cent of added water) than it is to describe milks as adulterated which are in reality genuine but poor.

It should always be borne in mind that added water, if present, may have gained access accidentally. Thus the following can be the cause of extraneous water in milk—

(1) Leaky coolers.
(2) Failure to drain tanks, churns, pipelines, etc., after washing.
(3) Freezing and thawing of milk without proper bulking.

In practice minute amounts of water are added from wet utensils, plant and bottles, but these normally do not reach a total of 1 per cent and so are not significant. Conversely there is always a minute loss of water due to evaporation especially in hot weather, and when milk is pasteurised by the batch or holder method. Under ordinary conditions neither is sufficient to affect the freezing point by a detectable amount and they probably about cancel each other.

Methods of detection of watered milk

Many years ago it was considered that added water could be detected almost with certainty from a determination of the solids-not-fat alone, when this figure fell below the agreed limit. It was later recognised that there are a few herds which give what has been called, for want of a better term, " abnormal " milk, having solids-not-fat much lower than usual. It is these milks which may cause difficulty and possibly some miscarriage of justice, if reliance be placed merely on the percentage of solids-not-fat in a particular sample. Some account is given below of the methods which have been adopted with the idea of overcoming this difficulty, and of the success which has been obtained.

(1) *The Vieth ratio*

Following the consideration of many thousands of analyses, Vieth enunciated the proposition that the ratio of the lactose, proteins, and mineral matter (ash) in a milk was 13 : 9 : 2, and that this ratio was very exact, varying little from milk to milk. As a result of the labours of Vieth and subsequent workers, among whom Richmond was prominent, it was discovered that this ratio holds good only for a " normal " or " usual " milk, and that in the case of a milk naturally low in solids-not-fat the relationship no longer holds, largely due to the fact that the lactose is deficient, whilst the proteins[1] and ash remain about the same. It follows that if, in the case of a milk low in solids-not-fat, the Vieth ratio is normal, added water is indicated, since water will reduce each of these constituents in the same proportion; whilst if the lactose only is low it may be assumed with every probability of correctness that the milk is genuine. This method has been used with considerable success over a number of years, and can always be applied usefully, where difficulty is experienced, as a confirmatory test.

(2) *Appeal-to-cow samples*

An appeal-to-cow sample is one which is taken after the cows have been milked at a supervised milking, under conditions which preclude the possibility of any kind of tampering and which ensure that the sample is a true indication of the quality of the milk given by the cows. The idea on which the assumed usefulness of the appeal-to-cow sample is based is that the milk from a herd of cows will vary little in composition in the corresponding milkings from day to day. The possible day-to-day variation in a herd is a measure of the usefulness of the appeal-to-cow method.

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[1] This fact led H. Hawley, Presidency Analyst of Madras, to propose a limit of 3·2 per cent of proteins (actually 0·5 per cent of nitrogen, but nitrogen multiplied by 6·38 gives the equivalent of proteins) as a criterion of genuine milk.

In the considered opinion of the revisers, the percentage of solids-not-fat in the milk of a herd of cows will not vary seriously from day to day, certainly not so seriously as to cause any miscarriage of justice where the appeal-to-cow sample is used as the final criterion. If a sample of milk is low in solids-not-fat by reason of natural deficiency, then the milk from the same cows will always show this tendency, and an appeal-to-cow sample will settle the point. It is useful and valuable to compare the Vieth ratio in the appeal-to-cow sample with that in the original deficient sample. To see clearly the importance of a combination of these two factors, let us imagine that a milk has been received which contains 8·2 per cent of solids-not-fat, and it is desired to decide whether this is adulterated or naturally deficient. The ash, proteins and lactose are determined and the Vieth ratio calculated. Where the latter is of the order 13 : 9 : 2 it is almost safe to hold the opinion that the milk is watered, whilst if the lactose is much lower than this ratio requires, the milk is probably genuine. In order to strengthen this opinion, an appeal-to-cow sample is taken. If the percentage of solids-not-fat in the appeal-to-cow sample is low—8·1 to 8·4 per cent—then it may be accepted without reserve that the original sample was genuine but poor. If, however, the appeal-to-cow sample contains about 8·7 to 8·9 per cent of solids-not-fat, the original sample was in all probability adulterated, and this opinion will be almost certainly correct if the Vieth ratio in the original and in the appeal-to-cow sample is the same or nearly the same.

For an incorrect opinion to be drawn from a consideration of the percentage of solids-not-fat and the Vieth ratio of two such samples, it would be necessary not only for the milk of the herd to vary in a most unusual manner, but that this variation should extend to the several ingredients of which the solids-not-fat are made up. Now when variation does take place in the milk of a herd, it is usually in two of the constituents. It is unlikely to the fringe of impossibility that exactly the same amount of variation will take place at one and the same time in the ash, in the proteins, and in the lactose.

(3) *The detection of nitrates*

Cows' milk is free from material traces of nitrates, even when the cows have had such salts administered to them medicinally. On the other hand, most natural waters obtained from wells contain traces of nitrates, which can be detected with ease. If, therefore, a milk is found to contain nitrates, it is a fair assumption that it is diluted with water containing nitrates, and the value of this assumption is increased if any water which might have been added is found to contain nitrates.

It cannot be too strongly urged, however, that the converse does not hold. A milk which does not contain nitrates is not necessarily genuine. Many public water-supplies, particularly those from mountains and moorlands (known as upland surface water), contain either no nitrates at all or so little that it would be quite impossible to detect them when added to milk. This test may, therefore, be regarded as a useful confirmatory test for the presence of water containing nitrates, but a negative result is of no value in deciding that a milk is genuine. Nitrates added to a milk disappear somewhat quickly. It is quite useless, therefore, to endeavour to detect nitrates in a sour milk, or even in one which is not quite fresh.

(4) *Calculation from the chemical constituents*

Mathieu and Ferré (1914) worked out a formula which denotes the sum of the weight of crystallised lactose and of the sodium chloride expressed as the (isotonic) equivalent of lactose. The value of this constant for most milks is said to lie between 74 and 79, and to be reduced below 74 when any considerable quantities of extraneous water are present.

The object of this method is to obtain a figure for the osmotic pressure of the milk by purely chemical means. The only substances present in a milk which exert any material influence upon the osmotic pressure (and therefore on the freezing point, which is a measure of this pressure) are the lactose and the soluble mineral salts. Now milks which are deficient in lactose usually contain a correspondingly high proportion of soluble salts, and the soluble salts consist for the most part of common salt and similar bodies, so that the common salt (really soluble chlorides calculated as common salt) may be expected to fall and rise as the lactose rises and falls. By combining, therefore, both these substances in one expression, a constant figure is to be expected.

This method has been examined by various workers (Mathieu 1916, Ferris 1917, Sirot and Joret 1919, Fonzes-Diacon 1919, Joret and Radet 1927) who have found this suggested constant to vary more than had originally been supposed. Figures varying between 69·2 and 82·8 have been reported.

Post (1926) introduced what he termed the " cryolac number ". This is an expression which is obtained by calculating the theoretical freezing point due to the lactose and chlorides (calculated as common salt), both of which are determined chemically. Fiehe and Kordatzki (1928) found that the cryolac number accounted for 75 per cent of the total freezing-point depression, and that it varied between 393 and 435 with a mean of 413.

The main point of the cryolac number would appear to be that it is determined by chemical rather than physical means. The determination of lactose and chlorine would certainly take much longer than a determination of the freezing point, and moreover the results, as they take into account only a portion of the substances on which the osmotic pressure depends—a portion which is not necessarily constant—are unlikely to be so valuable. On all grounds, the actual determination of the osmotic pressure by the freezing-point method must be considered greatly superior to a method giving a figure which may be proportional to it—the more certainly as in abnormal milks, which are, after all, the only cases where difficulty is likely to arise, this proportionality may no longer exist.

(5) *The refraction of the serum*

It seems to be a little doubtful when the refraction test was first proposed. Priority has been attributed to Utz and also to Villiers and Bertanet. It was first extensively used, however, by Leach and Lythgoe (1904). Other workers were impressed by the method, and it was examined at considerable length by Elsdon and Stubbs, who in a series of papers (1927, 1928a, 1928b, 1929, 1930b) gave very definite statements of the conclusions to which they were forced as a result of their experience. In their opinion the refraction test has not fulfilled the early anticipations entertained, nor substantiated the claims made for it.

There may be, of course, no direct mathematical relationship between the refraction of the serum and the percentage of solids-not-fat of any one milk, but the results of many workers show that when the solids-not-fat are high, a high refraction is to be expected, and when the solids-not-fat are low, from whatever cause, a low refraction is to be expected. The refractometer will not, of itself, decide between a naturally poor milk and a watered milk having the same composition, since, in general, the refractions will be of the same order.

The refraction of ordinary genuine fresh milks, using the copper-sulphate serum method at 20° C, will vary between 37 and 39 degrees on the Abbé immersion scale. An occasional sample will be found which has a refraction of less than 37, when, if the solids-not-fat are low, this may be due to deficiency in lactose in an abnormal milk or to added water. This point can only be decided by the application of other and more specific tests.

Mitchell and Frary (1948) have expressed the opinion that the refractive index method cannot detect less than 5 per cent added water in milk using the present A.O.A.C. standards, and suggest raising the lower limits for n and serum ash.

Occhialini (1947) has proposed to use the density of the acetic acid serum (Q) and the chloride content (C) to detect adulteration. His formula is

$$Q + 3{\cdot}85\ C = 35{\cdot}1$$

In genuine milks this formula gives values from 34·08 to 36·98. He claims to have detected 5 per cent added water in 82 per cent of cases and that this method is not invalidated by isotonic adulteration as is the freezing-point test.

(6) *Spectrometric measurements*

Recently the spectrometer has been suggested as a means by which the addition of water to milk may be detected. Research on this point was undertaken by an expert in spectroscopy who, in a private communication, gave the revisers his opinion on the possible utility of the method. He considered that the detection of 10 per cent of ordinary water added to a milk is possible by this means. The subject is, however, in its infancy and more work is needed by independent observers before the value of the test can be definitely settled. It should be emphasised at the outset that the method can only be of limited value for proving the *purity* of a milk, since the addition of a pure water, such as rainwater or many upland surface waters, would not be indicated. It may be of value for proving the adulteration of a given sample of milk, but can never be of final value in proving that a milk is genuine.

(7) *The freezing point of milk*

When the concentrations of two solutions which are separated by an animal membrane differ, there is a tendency for the solvent to pass until the osmotic pressures on both sides are the same. In the animal body we have just such conditions, and it is therefore to be expected that the main body fluids will have similar osmotic pressures, and that this will apply particularly in the case of the blood and the milk, which are so intimately connected. Further, it can be shown that the freezing-point depression of a solution can be used as a measure of its osmotic pressure, and we have, therefore, in this method a ready means of determining such pressures.

In 1892 Dreser drew attention to the comparative constancy of the osmotic pressure of the blood and milk of cows, as determined by means of the freezing-point method, and his conclusions have been confirmed by subsequent investigations. The comparative constancy in the freezing point of milk was, therefore, regarded by Beckmann and Jordis (1895) as being likely to be valuable in the detection of added water.

For all ordinary milks there is now no question but that the range of freezing points from herd to herd, or even from animal to animal, is extremely small. It follows, therefore, that for normal milks the detection of five per cent (or even less) of added water is comparatively simple, With regard to abnormal samples, in every case so far investigated the abnormality has taken the form of a greater depression rather than a smaller, so that although the addition of a little water to an abnormal milk might pass on the evidence of the freezing point alone (even this possibility is much reduced by the taking of appeal-to-cow samples), it is unlikely, in fact almost impossible, that a genuine milk would be suspected of being watered.

Calculation of the amount of added water

The actual amount of added water is best calculated from the freezing point as described on p. 140. When this method is not available, the amount can be calculated from the solids-not-fat content by the equation—

$$\text{Percentage added water } \frac{S'-S}{S'} \times 100$$

where S' represents the percentage of solids-not-fat in the original milk and S that in the watered milk. When, as is often the case, the composition of the original milk is unknown, the limit of the Sale of Milk Regulations, 8·5 per cent, may be substituted for S', when the equation becomes—

$$\text{Percentage added water } \frac{8{\cdot}5-S}{8{\cdot}5} \times 100$$

This is the formula usually adopted in the certificates of public analysts. In general it gives a lower figure for added water than the amount actually present.

Calculation of fat deficiency

The percentage of the total fat present which has been abstracted may be calculated from the formula—

$$\text{Percentage fat abstracted} = \frac{F'-F}{F'} \times 100$$

where F' represents the percentage of fat in the original milk and F that in the milk under examination. In general the exact amount of fat in the original milk will not be known. In such cases the percentage abstracted may be calculated from the limit of the Sale of Milk Regulations, 3·0 per cent, when the equation becomes—

$$\text{Percentage fat abstracted} = \frac{3{\cdot}0-F}{3{\cdot}0} \times 100$$

This formula gives a minimum percentage of fat abstracted. The figure thus calculated is almost always seriously below the truth; the probable amount can be calculated by substituting 3·75 for 3, or, better still, the monthly average figure given in Table 3.17 on p. 74 for the month in which the analysis is made.

The action of cold on milk: effect on composition

When milk is exposed to a low temperature it freezes partially. As with other aqueous solutions, the freezing point is, as we have seen in Chapter 5, below that of water and is about —0·55° C (31° F), estimated in the Beckmann apparatus. The frozen portion has not the same composition as the milk from which it was prepared, but contains a larger quantity of water. Owing to the facts that milk has not a point of maximum density, and that it does not freeze as a homogeneous substance, ice never forms in a solid layer on the surface. The following analyses (Table 7.2) will show the composition of the frozen and liquid portions respectively.

It is seen that no appreciable difference between the ratio of the sugar to the protein and the ash is found in the two series of analyses, showing that no separation of any constituent except water takes place during freezing. It is seen also that the greater the percentage of ice separated, the more dilute is the solution from the re-melted " ice "; this is best seen by calculating the solids-not-fat content (Table 7.3).

Table 7.2—Composition of the solid and liquid portions of frozen milk

	Liquid portion	Frozen portion
	Percentage of ice formed, 1·2 per cent	
Specific gravity	1·0320	1·0245
	Per cent	Per cent
Water	86·72	91·63
Fat	4·11	2·40
Protein	3·56	2·40
Sugar	4·87	3·05
Ash	0·74	0·52
	Percentage of ice formed, 2 per cent	
Specific gravity	1·0330	1·0190
	Per cent	Per cent
Water	87·10	91·83
Fat	3·87	2·56
Protein	3·21	2·28
Sugar	5·08	2·89
Ash	0·74	0·44
	Percentage of ice formed, 2·25 per cent	
Specific gravity	1·0330	1·0180
	Per cent	Per cent
Water	87·21	92·46
Fat	3·57	2·46
Protein	3·50	1·96
Sugar	4·98	2·72
Ash	0·74	0·40

Table 7.2—Composition of the solid and liquid portions of frozen milk—*contd.*

	Liquid portion	Frozen portion
Percentage of ice formed, 10 per cent		
Specific gravity	1·0345	1·0090
	Per cent	Per cent
Water	85·62	96·23
Fat	4·73	1·23
Protein	3·90	0·91
Sugar	4·95	1·42
Ash	0·80	0·21

Table 7.3—Composition of "ice" separated from frozen milk

Percentage of "ice"	1·2	2·0	2·25	10·0
Solids-not-fat	6·17	5·61	5·08	2·64
Equal to percentage of added water (approx.)	30	38	43	70

As all these samples were taken from churns in which milk was brought up to London, the percentage of ice may be taken as indicating roughly the temperature below freezing point to which the milk was exposed, the time of exposure to the low temperature having been approximately the same in all cases. It appears that the lower the temperature to which milk is exposed, the more dilute will be the solution on re-melting the ice.

Composition of re-melted frozen milk

The difference in composition between frozen and unfrozen milk may have some importance, should samples be taken under the Food and Drugs Act in very cold weather; should an excessive proportion of ice be present in the portion sold to the inspector, the sample may, though originally genuine, have the composition of watered milk.

Vieth has recorded an interesting experiment on the freezing of milk: two gallons of milk were exposed to a temperature of —10° C (14° F) for three hours; longer time than this did not render any more milk solid. Ice was formed on the bottom and sides of the vessel employed to contain the milk, and a funnel-shaped cavity in the centre was filled with liquid. The ice was found to consist of two layers, one of cream, and the other of skim-milk; these were separated as completely as possible and the liquid portion also poured off.

The results of analysis were—

Table 7.4—Composition of portions of a sample of frozen milk (*Vieth*)

	Frozen portion		Liquid portion
	Cream	Skim-milk	
Proportion	8·8 per cent	64·7 per cent	26·5 per cent
Specific gravity	1·0100	1·0275	1·0525
	Per cent	Per cent	Per cent
Water	74·44	92·10	80·54
Fat	19·23	0·68	5·17
Protein	2·64	2·80	5·38
Milk-sugar	3·33	3·95	7·77
Ash	0·52	0·60	1·18
	100·16	100·13	100·04

Another experiment gave almost identical figures.

It is probable from these experiments that milk exposed to a temperature of —10° C will always yield a liquid portion having the composition given above. The figures also show that milk cannot be frozen into blocks, from which pieces can be cut off and melted for use, without modifying the composition to a serious extent.

Richmond had the opportunity of examining three samples of milk which had been frozen for transport and re-melted (Table 7.5). The samples were taken under such conditions as would represent the retailing of the milk—

Table 7.5—Composition of three samples of frozen milk

	(1)	(2)	(3)
Specific gravity	1·0385	1·0270	1·0325
	Per cent	Per cent	Per cent
Total solids	13·60	10·13	11·68
Fat	3·29	2·70	2·86
Ash	0·84	0·62	0·74
Solids-not-fat	10·31	7·43	8·82

No. (1) has the composition of concentrated milk, (2) of a watered milk, and (3) of a slightly skimmed milk.

Davidow (1949) suggests that under certain conditions part of the water may form a solution of acids and salts which precipitates the protein. If milk is agitated during freezing it is normal after thawing. Milk frozen and held at —25° C shows no precipitation, but at higher temperatures precipitation takes place. Corley and Doan (1940) have studied the possibilities of concentrating and freezing milk (—17·8° C) as means to its preservation. Pasteurisation at 82° C for 30 min. gave a cooked flavour with stainless steel equipment; copper plant induced a tallowy flavour, especially in homogenised milk and low-temperature pasteurised milk. Trout (1941b) has reported that homogenised milk[1] freezes rather more rapidly than ordinary milk. The frozen plug is harder, but " flakiness " is never found after melting. The thawing of frozen homogenised milk tends to give a clear plug and a watery upper layer. Roadhouse and Henderson (1940) have frozen milk at —25° F (—31·7° C) and held it at —5° F (—20·6° C). Raw milk became oxidised after 2 weeks, but pasteurised milk, cream and evaporated milk were palatable up to 6 months.

Babcock *et al.* (1946, 1947a, 1947b) have published a series of papers dealing with the stability and keeping quality of frozen homogenised milk. They have found (1948a) that changes in flavour of the thawed product are similar to those in unfrozen homogenised milk. Milk of good quality may be held at 2° C for up to 120 hrs. and still give a good frozen product (1948b). They have also shown (1949) that sodium citrate at 2 g/litre is a good stabiliser. Separation did not occur in the thawed product until after 145 days' storage and flavour deterioration until after 105 days' storage.

Bell (1948) has studied changes in O/R potential and ascorbic acid content in frozen milk and concludes that low E_h values brought about by addition of ascorbic acid can retard but not prevent the development of oxidised flavour. Low potentials did not appear to stabilise the ascorbic acid.

Bell and Mucha (1951) have suggested that milk should be pre-heated to at least 71° C for one minute before concentration and final freezing. The best results are obtained when 50 mg per litre of ascorbic acid are added.

Wildasin and Doan (1951) have discussed a number of factors which affect the stability of frozen concentrated milk. The addition of sufficient sucrose extends the satisfactory frozen storage period. The effect of heat on the protein stability of skim milk when stored frozen cannot be predicted unless the previous history of the product is known. The eu-globulin appears to be an important factor in this respect. The protein floc appearing in stored frozen milk consists mainly of casein.

The concentrated milk, especially when the solids content is high, should be frozen as soon as possible after condensing. Clarification does not appear to result in the retarding of the flocculation of the protein.

Attempts have been made to introduce frozen, or partially frozen, milk into the English market from Holland and other countries. The figures given in this section show what may sometimes be the composition of such milk as retailed, unless extreme care be taken in melting the imported product.

(2) ABSTRACTION OF FAT

There is, unfortunately, no single test which can be applied to milk which will give definite information as to whether fat has or has not been abstracted from a given sample of milk. The information can only be obtained by methods of deduction. How far these methods can be relied upon can be judged only by a careful consideration of the whole of the evidence which can be adduced in any given case.

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[1] Defined on page 182.

The fact that the mixed milk of a herd of cows contains less fat than the general average is no evidence that fat has been abstracted—the mere fact of there being an average implies that some milks contain more fat than others; indeed, it is roughly true that the number of different herds giving milk having a lower fat content than the average will be about the same as those giving milk containing a higher fat content than the average.

Reference to the section dealing with the likely day-to-day variations in the fat content of milk (p. 81) will show that, although this figure can be affected by quite a number of factors, it is, nevertheless, often possible to ascertain whether a poor fat content is due to natural causes or to adulteration.

If the milk of a herd of healthy cows under normal conditions contains less than 3·0 per cent of fat, suspicion at once naturally arises that the milk is not genuine. If the sample is one of evening milk the suspicion becomes a very strong one, as evening milk usually contains more fat than the corresponding morning milk, and it is a very rare occurrence indeed for the evening milk of a herd of cows to contain less than 3·0 per cent of fat. The following table contains the percentages of fat found in a milk alleged to be the product of a herd of ten cows; those on the Wednesday were samples purchased in the ordinary way, those of Thursday were appeal-to-cow samples—

	Morning	Evening
Wednesday	3·4	2·7
Thursday	3·3	3·9

In this case the opinion expressed was that the Wednesday evening milk was not in the condition in which it was given by the cows.

It is most unlikely that a milk which contains much less than 3·0 per cent of fat (say 2·8 per cent or less) will be subject to an increase of more than 0·5 per cent in two days, and where the variation is more than this it will, in general, be safe to express the opinion that such a variation is not due to natural causes (cf. p. 81).

Where it is contended that the poorness of a given sample of milk is due to some sudden change in the climatic conditions, it frequently happens that the results of analysis of other samples of milk, taken in the same district and on the same day, can be used to refute this line of argument. The revisers have never met a batch of milks taken on the same day in the same district the average percentage of fat of which has been materially below the average for that district, which fact strongly suggests that sudden extremes of heat and cold do not exert any serious influence upon the composition of the milk of cows subjected to such conditions. In summarising the evidence on this subject, whilst agreeing that the determination of the abstraction of fat is more difficult than the determination of the addition of water and is not subject to such exactitude, the revisers are of the opinion that gross adulteration, either by abstraction of fat or the addition of separated milk (which comes to the same thing), may be detected, providing that the whole of the circumstances and the available evidence are taken into consideration.

(3) MISCELLANEOUS TESTS

The absence of carotene from sheep's milk permits the distinction of goats' milk from sheep's milk, and the detection of the adulteration of sheep's milk by cows' milk in cheese-making. The absorption method detects 20 per cent or more of cow butter fat (Anon. 1940). Rössler (1948) favours a slide coagulation test for detecting cows' milk in human milk, using a rabbits' anti-serum. Sastry and Dastur (1947) have studied several methods for detecting the presence of skim-milk powder in whole milk and obtained promising results with tests based on dry residue after centrifuging, flavour development and rennet coagulation. It has been reported (Anon. 1947) that addition of reconstituted milk to fresh milk can be detected by " burnt odour " and by the effect of dilution on rennet clotting.

CHAPTER 8

CREAM

Cream was defined by the Public Health (Milk and Cream) Regulations, 1912, as "that portion of milk rich in milk fat which has risen to the surface of the milk on standing and has then been removed, or which has been separated from milk by centrifugal force"; whilst the Food and Drugs Act, 1938, defines it as "that part of milk rich in fat which has been separated by skimming or otherwise".

Qualitatively, cream has the same composition as milk; quantitatively, it contains a higher proportion of fat, the other constituents being correspondingly depressed.

It has been claimed that cream contains a larger proportion of solids-not-fat to water than the milk from which it was derived, and various explanations of this have been put forward. Thus a membrane round each fat globule has been alleged to exist by some (e.g. Storch and Béchamp); others have considered that the proteins are concentrated in the aqueous layer formed round each globule by surface tension. Richmond's experiments indicated that the ratio of solids-not-fat to water in cream is the same as that in milk, and Weibull and Smith and Leonard have confirmed this conclusion. It is true that in some cases a distinctly higher ratio has been found, but it has been noticed that in these cases ample opportunity for evaporation of the water had been afforded, either by leaving the cream on the surface of the milk for some length of time in a dry atmosphere, or by pasteurising it, without any precautions to prevent evaporation; indeed, evidence of evaporation has been obtained by noting the quantity of cream before and after pasteurising. In cases where precautions have been taken to prevent evaporation, no evidence of a higher ratio has been obtained.

Table 8.1—Fat in fresh cream, 1908 to 1936

Fat per cent	No. of samples	Fat per cent	No. of samples
Below 30	12	48·0 to 49·9	34
30·0 to 31·9	3	50·0 ,, 51·9	30
32·0 ,, 33·9	4	52·0 ,, 53·9	21
34·0 ,, 35·9	4	54·0 ,, 55·9	22
36·0 ,, 37·9	6	56·0 ,, 57·9	11
38·0 ,, 39·9	6	58·0 ,, 59·9	9
40·0 ,, 41·9	9	60·0 ,, 61·9	9
42·0 ,, 43·9	9	62·0 ,, 63·9	5
44·0 ,, 45·9	14	64·0 ,, 65·9	3
46·0 ,, 47·9	18	Over 66·0	7

The complete range was 8·0 to 76·0 per cent.

From the very nature of the product it is useless to attempt to give an average composition. The cream which rises to the surface of milk on standing may contain as little as 10 per cent of fat, but cream for the market is now seldom, if ever, prepared by this method. Modern separators can be arranged to yield a cream containing anything from 10 to 65 per cent of fat, but a normal amount is from 45 to 55 per cent. Table 8.1 gives the amounts of fat found in 236 samples bought as " cream " during the years 1908 to 1936.

The Ministry of Food permitted the sale of cream during the summer of 1951. Standards were—

Single (or " coffee ") cream (commonly homogenised)	18 per cent
Double or thick cream	48 ,,
Canned cream	23 ,,

Johnson and Fouts (1943) have found that sodium carbonate is generally more effective than calcium hydroxide in neutralising saturated fatty acids in cream (butyric to stearic and oleic). Up to 0·15 per cent acidity the acids were equally easily neutralised, but from 0·2 to 0·5 per cent butyric and caproic acids were more readily neutralised. Oleic was more resistant than the other acids, and calcium hydroxide was somewhat more effective than sodium carbonate as a neutraliser.

Clotted cream, or cream prepared by the system practised in Devonshire and Cornwall,[1] was examined regularly in the Aylesbury Dairy Company's laboratory during the years 1886 to 1915. The following are the average results, together with the maxima and minima found—

Table 8.2—Composition of clotted cream

	Water per cent	Fat per cent	Ash per cent	Solids-not-fat per cent
Average	34·26	58·16	0·60	7·52
Maximum	44·84	71·37	1·17	11·70
Minimum	21·08	44·29	0·42	5·03

It is seen that the ratio of solids-not-fat to water is very much higher in clotted cream than in milk, due to the evaporation which takes place from the surface during heating, and roughly speaking, the ratio of solids-not-fat to water is double the average ratio in milk.

The ratio of ash to solids-not-fat is very nearly the same in clotted cream as in milk; actually it is slightly lower. This is partly if not entirely due to the fact that, on heating milk, certain salts of calcium, probably phosphate and citrate, are deposited, leaving a smaller proportion in the milk and also in the cream derived from it.

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[1] The essential details consist in allowing the cream to rise to the surface of the milk, heating on a water-bath, gradually raising the temperature nearly to boiling, then allowing the milk to cool slowly, and removing the thick layer of cream.

The thickness of cream

The thickness is the factor by which cream is usually judged when used for direct consumption. This can be estimated quantitatively by the method generally employed for the determination of " viscosity ", i.e. noting the time taken for a given volume of cream to flow through a tube of constant size. The viscosity of a liquid depends on the internal friction, i.e. the friction of molecules passing each other, but the viscosity or internal friction of cream is not quite of the same order as that of a homogeneous liquid; in the latter case, the molecules are of equal size (or nearly so), and very small in comparison with the diameter of the tube through which the liquid passes. The viscosity of cream depends on two factors—the internal friction of the very small molecules of the milk serum, and the friction between the comparatively large fat globules.

As the fat globules have an appreciable size compared with the size of the tube, we cannot expect the laws to be of the same kind as those governing the viscosity of a liquid composed of molecules which are, comparatively, minute in size. The actual and relative size of the globules will also have considerable influence; thus, if we have two creams identical in chemical composition, in one of which the relative size of fat globules is much larger than in the other, the " viscosities " will differ.

It is not possible to measure the thickness of cream by making a determination of the percentage of fat in a sample. It is possible, however, to make a comparison of creams which contain globules of relatively the same size. For instance, if cream be diluted with separated milk, which is practically free from fat, the thickness can be deduced by making determinations of fat. The law connecting thickness or viscosity and amount of fat is expressed by the following empirical formula—

$$V = 10^{xF^3},$$

where V = the viscosity,
F = the volume of fat in 100 volumes of cream,
and x = a factor dependent on the units in which the viscosity is expressed, and on the relative size of fat globules.

According to Mohr and Wellon (1948) the globules in cream of more than 75 per cent fat are packed so tightly together that they deform each other. They suggest that this effect is the first stage in the Alfa butter-making process, in which phase inversion occurs in 80 per cent cream. Viscosity increases rapidly when the fat percentage exceeds 60 over the temperature range 104°–140° F (40°–60° C). Smith and Doan (1948) have found that the increased viscosity of re-separated chilled pasteurised cream is not controlled by separator speed. They suggest that the warming and cooling processes involved are really responsible and not the re-separation as such. The well-known increase in viscosity obtained by warming and cooling pasteurised cream has been studied by Wiese *et al.* (1939), in an attempt to find out which of the constituents is responsible. None of the skim components appeared to affect the cream in this way. The globule membrane material was the most effective in this respect.

Sommer (1943) has discussed the whipping of low fat cream. Rigidity is controlled by the contiguity of the fat and dispersed air cells. Calculation shows that if all the particles concerned were spheres of equal size, contiguity would be reached at an over-run of 118 per cent in 32 per cent cream and 184 per cent in 19 per cent cream. Whipping breaks up the air cells and clumps the fat, but optimum whip is reached when the fat achieves contiguity with the air cells, this occurring before equality of size is attained.

Artificial thickening of cream

Cream has been artificially thickened by the addition of various foreign substances; thus, gelatine, isinglass, agar, and substances of like nature have been employed, but without great success, as the cream thus treated has an appearance markedly different from that of genuine cream. The following method, due to Stokes, may be applied to detect gelatine in cream. To 10 g (approximately) of cream add 25 ml of water and 2 ml of Wiley's acid mercuric nitrate solution (p. 370), and shake well; filter through a dry filter. In the presence of much gelatine, the filtrate cannot be obtained clear, and it is not essential that it should be so. On adding a saturated aqueous solution of picric acid, a yellow precipitate is formed in the presence of gelatine; if the quantity of gelatine be small, the precipitate does not form at once, but the solution becomes turbid, and precipitates after a lapse of some minutes. Seidenberg finds that sour cream gives a precipitate with picric acid, which can be distinguished from that given by gelatine by its insolubility in hot water; the hot water solution can be filtered, concentrated, and re-tested with picric acid for gelatine. Starch which has been gelatinised by heating has also been used; this, of course, is readily detected by the characteristic blue coloration given with tincture of iodine.

Of comparatively recent introduction is " viscogen ", which is a solution of lime in cane-sugar syrup; the addition of a small amount of this substance has a remarkable effect in increasing the thickness of cream. It is sold under various proprietary names.

The presence of viscogen may be detected by testing the cream by one of the methods (p. 413) for the detection of cane sugar; the amount of ash will be raised, and the ratio of lime to phosphoric acid in the ash will be higher than 17 : 23. It is usually added in quantities of about 0·5 per cent; this amount increases the solids-not-fat by about 0·2 per cent of cane sugar, the ash by about 0·04 per cent, and raises the ratio of lime to phosphoric acid to about 1 : 1.

As homogenised cream will not whip, it is not uncommon to add gelatine, agar, or gum tragacanth for the purpose of making a fairly permanent foam when the cream is whipped. Of these, gum tragacanth added in the proportion of 0·1 per cent is the most effective. A careful microscopic examination of the cream, after the addition of a little iodine solution, will reveal the presence of particles of gum tragacanth, in which starch granules can be detected. Agar, which has also been used as a thickening agent, gives Cayaux's resorcinol test for sucrose, but not any of the other tests.

Bolton and Revis test for agar by diluting 50 ml of cream with 100 ml of water, adding 5 ml of 10 per cent calcium chloride solution, boiling and filtering; to the cooled filtrate one-half to two-thirds of strong alcohol is added, the precipitate is filtered off and boiled with a small quantity of water till no more dissolves, filtered hot and evaporated to 5 ml; in the presence of agar the solution gelatinises. If gelatine be present, it must be removed by adding tannin to the filtrate, preferably evaporated to 25 ml till no more is precipitated. After cooling below 60° a little white of egg is added, and the whole heated in boiling water for 30 minutes, and the filtrate evaporated to 5 ml as before.

Cream has also been thickened by adding a strong solution of casein in alkalis' condensed milk, or milk powder. These may be detected by the solids-not-fat being appreciably higher than the figures given on p. 179, and also by the aldehyde figure, calculated to the cream devoid of fat, being much above 22°.

Homogenisation of milk

In the equations given on p. 185 the fat globules are considered as being free from any condensed layer, but this is not the case, as the surface energy of small globules condenses round them a layer of protein, etc., which may, for physical considerations, be included in the globule; this will decrease the value of d_s—d_f (see "The rising of fat globules and separation of cream", below) and will retard, and in extreme cases stop, the rising of very small globules. In the case of the globules of cows' milk, the influence of the layer is, though not absolutely negligible, sufficiently small to be left out of consideration. When, however, the globules of fat are reduced to a diameter below that of the smallest naturally-occurring globules, it becomes of more importance, and the rate of rising of cream is much less than that indicated by the formulae.

By forcing milk, heated to such a temperature[1] that surface energy is reduced to a minimum while chemical change in the milk is prevented, under a high pressure through very small openings, the fat globules are reduced to a very small size. The condensed layer bears such a relation to the globule that the cream rises with extreme slowness and, practically speaking, remains mixed with the milk. This process is termed *homogenisation*. Owing to the fact that the condensed layer is held so firmly by the great surface energy of small particles, it is impossible to churn milk or cream that has been homogenised; as the effective diameter of the globules is increased by the condensed layer, homogenised milk and especially cream are thicker for the same percentage of fat than fresh milk or cream. Surface energy, and consequently the thickness of the layer, vary considerably with temperature; for this reason the thickness of homogenised cream varies more with temperature than the thickness of ordinary cream.

Homogenising decreases the curd tension of milk proportionately up to a working pressure of about 2,000 lb., but higher pressures produce little further change. The reduction is enhanced by increase in temperature. Homogenisation accelerates the initial digestion of milk, the curd having a four- or five-fold increase in surface area. It also makes the milk about 10 times more resistant to oxidation catalysed by copper. However, homogenised milk is twice as susceptible to light-catalysed oxidation as ordinary raw milk. There is apparently no difference between the keeping qualities of ordinary and homogenised milk (Kelly 1940, 1942a).

Weinstein and Trout (1951) have reported recent studies on what is called the "solar-activated" flavour of homogenised milk. This taint is also referred to as "burnt protein" and "burnt feather". They have found that whereas homogenised milk from all cows on dry feed was susceptible to the development of this sun-activated flavour, only 30 per cent of the milks from individual cows on pasture failed to develop this taint after pasteurisation, homogenisation and exposure to the sun for 30–60 minutes. There was apparently no correlation between breed, stage of lactation, and fat percentage and susceptibility of the milk to develop this taint. Some cows on summer pasture yielded milk which developed a nauseating flavour quite distinct from the true sun-activated flavour. This flavour appears to arise from an oxidation process and the addition of ascorbic acid has no effect upon its development. It can be prevented, however, by the addition of 25 mg per litre of nordihydroguaiaretic acid.

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[1] The temperature should not exceed 60° C, as the mechanical work done in forcing the milk through small openings is partly converted into heat, which raises the temperature of the milk some degrees.

Alpha-tocopherol and hydroquinone, separately or in conjunction, did not protect homogenised milk completely against developing this taint. If the homogenised milk were treated with hydrogen peroxide to destroy the naturally occurring ascorbic acid prior to pasteurisation and homogenisation, the milk did not develop this flavour when exposed to sunlight. Heat treatment for five minutes at 176° F did not retard or prevent the development of the flavour.

The surface area of the fat globule appears to be a factor controlling off-flavour development, but there is no definite correlation between the taint and a positive Kreis reaction. These workers were unable to identify the constituent affected.

Ashworth (1951) has ingeniously used turbidity measurement to measure the efficiency of homogenisation. The " K value " or ratio of optical density to the fat content was found to be (in the units used) 1·0 for unhomogenised milk, and about 2·2 for properly homogenised milk.

Trout (1948) has compiled a review on the nutritive value of homogenised milk.

Canned cream

Canned cream, on the average, contains about half the amount of fat usually found in fresh cream. Sometimes the percentage of fat is given on the label, at other times it is not, but it is doubtful whether most purchasers are aware of the great difference in composition which usually exists between canned and fresh cream. Seventy-eight samples of canned cream examined between the years 1926 and 1936 gave the following results—

Table 8.3—Fat in canned cream, 1926 to 1936

Percentage of fat	No. of samples		
	1926–1930	1931–1935	1936
19·0 to 19·9	2	7	0
20·0 ,, 20·9	5	5	3
21·0 ,, 21·9	4	3	2
22·0 ,, 22·9	2	3	0
23·0 ,, 23·9	2	3	1
24·0 ,, 24·9	6	5	0
25·0 ,, 25·9	11	3	0
26·0 ,, 26·9	3	3	0
27·0 ,, 27·9	3	0	1
Totals	38*	32	7

* In addition one sample contained 39·9 per cent of fat.

Milk and cream sterilised at 250° F retain their flavour after a year's storage. A fat content of 25–30 per cent appears to be best (Anon. 1949).

Jack and Brunner (1943) have shown that it is possible to measure the proportion of solid fat in a sample of cream by simple calorimetry. The specific heat of milk fat is 0·5 and the latent heat of fusion 19·5 cal. per g. If H is the heat required to raise the temperature of 1 g of cream from T_1 to T_2 (the fat being completely melted at T_2), then the percentage of solid cream is

$$\frac{H-0{\cdot}5\,(T_2-T_1)}{19{\cdot}5} \times 100$$

The storage of frozen cream has been studied by Trout (1942) who concludes that a 50–60 per cent fat cream of good quality and flavour, low acidity without neutralisation, and absence of copper is necessary for long-keeping quality at —5° to —10° F (—20·6° to —23·3° C). Pasteurisation at 185° F (85° C) for 5 min. appears to be best. Trout was unable to devise any foolproof safeguard against " oiling-off ". Stull *et al.* (1947, 1948b) have studied the anti-oxidant properties of nordihydroguaiaretic acid for frozen cream. A concentration of 0·00125 to 0·005 per cent is claimed to retard the onset of oxidised flavour in unsweetened frozen cream for 11 months.

Artificial cream

Artificial cream is defined in the Food and Drugs Act, 1938, as " an article of food which, though not cream, resembles cream and contains no ingredient which is not derived from milk except water ". Its distinction from natural cream may not be easy, but in those cases where the constituents are not present in the proportions natural to cream, no difficulty should be found. The determination of the freezing point is valuable, but should be carried out on the serum (separated by centrifugal means), as the cream, when cooled, becomes too stiff for adequate stirring. F. W. Richardson (1928, 1933) has suggested two miscibility tests which he considers will distinguish artificial cream from natural cream. Such tests are sometimes useful, but a considerable amount of experience is required before they can be used with certainty. A better name would have been " reconstituted " cream.

Synthetic cream

Synthetic cream is similar in appearance to artificial cream, but is made by the emulsification of margarine or other non-milk fats. Its detection is based on an examination of the fat. It is perhaps unfortunate that the description " artificial cream " was given to the substance named in the Act.

The rising of fat globules and separation of cream

The globules of fat rise through the milk because they are lighter than the milk serum. If we have a globule of radius r, and density d_f, in milk serum of density d_s, the force impelling it to rise is given by

$$F = \frac{4}{3}\pi r^3\,(d_s - d_f)g$$

where g is the acceleration due to gravity.

The globule does not, however, rise freely. The resistance to its motion is given by Stokes's law, which may be written

$$F_R = 6\,\pi r \eta v$$

where v is the velocity at which the globule is moving, and η is the viscosity of the serum.

Then the net force acting upwards on the globule may be written

$$F - F_R = \frac{4}{3}\pi r^3 (d_s - d_f)g - 6\,\pi r \eta v$$

This force causes the globules to accelerate according to the equation

$$\left(\frac{4}{3}\,\pi r^3 d_f\right)\frac{dv}{dt} = \frac{4}{3}\pi r^3 (d_s - d_f)g - 6\,\pi r \eta v$$

or

$$\frac{dv}{dt} = \frac{d_s - d_f}{d_f}\,g - \frac{9}{2}\frac{\eta v}{r^2 d_f}$$

Putting

$$\frac{d_s - d_f}{d_f}\,g = a \quad \text{and} \quad \frac{9\eta}{2\,r^2 d_f} = b$$

we get

$$\frac{dv}{dt} = a - bv$$

Integrating this gives

$$v = \frac{a}{b}(1 - e^{-bt})$$

For large values of bt, e^{-bt} is so small as to be negligible, and the expression then becomes, substituting the values of a and b,

$$v = \frac{2}{9}\left(\frac{d_s - d_f}{\eta}\right) g r^2$$

It is evident that since b is inversely proportional to the square of the radius of the globule, bt rapidly becomes large for quite small values of t, and the globule soon takes up its steady velocity given by the above equation. The time taken for a globule to pass through a given layer of milk is, therefore, inversely proportional to the square of its radius.

If the globule is acted upon by a centrifugal force the acceleration due to gravity g must be replaced by the expression $\frac{\pi^2 V^2}{900}\,z$ where V is the velocity of the centrifuge in revolutions per minute, and z is the distance of the globule from the axis of rotation.

It is evident that the speed of the globule cannot be constant when subjected to centrifugal force, as the force tending to move it itself varies with the position of the globule.

The full equation of motion under these conditions is (remembering that $v = dz/dt$)

$$\frac{d^2 z}{dt^2} = \frac{d_s - d_f}{d_f}\,\frac{\pi^2 V^2}{900}\,z - \frac{9}{2}\,\frac{\eta}{r^2 d_f}\,\frac{dz}{dt}$$

Putting $$\frac{d_s - d_f}{d_f}\,\frac{\pi^2 V^2}{900} = c^2 \quad\text{and}\quad \frac{9}{2}\,\frac{\eta}{r^2 d_f} = b,$$

the solution to this equation is

$$\frac{z}{z_0} = e^{\frac{-bt}{2}}\;\frac{b}{\sqrt{b^2 + 4c^2}}\sinh\left(\sqrt{b^2+4c^2}\right)\frac{t}{2} + \cosh\left(\sqrt{b^2+4c^2}\right)\frac{t}{2}$$

where z_0 is the distance of the globule from the axis of rotation at time $t = 0$.

Where b is large in comparison with c, and this may be seen to be true for most practical cases, the above expression reduces, for large values of $\left(\sqrt{b^2+4c^2}\right)\frac{t}{2}$, to

$$\frac{z}{z_0} = e^{\frac{c^2}{b}t}$$

or

$$t = \frac{4050\eta}{(d_s - d_f)r^2\pi^2 V^2}\log\frac{z}{z_0}$$

which expresses the time taken for a globule to pass from any point in the separator to any other point, provided the serum is at rest, and the globule travels radially.

This is not the case in modern separators where the milk runs in continuously, and terms expressing the rate of flow of milk, and the shape of the separator, must be introduced. The resulting equations are so complex that it would serve no useful purpose to deduce a general equation.

Whatever the form of equation suited to any particular separator, the time taken by a globule to pass through a given space will always be proportional to the square root of the cube of the radius, and as the number of gallons per hour passed through the separator will be inversely proportional to the time, it follows that for each size of fat globule there will be a limit where its velocity against the stream of milk will be equal to the velocity of the stream itself, and all globules smaller than this will pass out with the separated milk. If we assume that the total weight of fat globules of any size is equal to the total weight of fat globules of any other size, it follows that the amount of fat in the separated milk is proportional to the cube root of the square of the number of gallons per hour. The coefficient of viscosity, and also the value of the factor $(d_s - d_f)$, vary with the temperature, and consequently the viscosity of the fat globules and the amount of fat in the separated milk.

The relative proportions of the cream and skim-milk will also affect the percentage of fat in the separated milk, as not only is the rate at which milk travels towards the separated milk outlet affected, but any resistance to the exit of cream causes the fat globules to touch each other, and interferes with their free motion.

Upon these considerations, Richmond worked out a formula to give the percentage of fat in the separated milk—

$$f = a \times b^{(40-t)}\frac{38}{t} \times c^{F} \times \frac{m^{\frac{2}{3}}}{v^2},$$

where f = percentage of fat in separated milk,
F = ,, ,, cream,

t = temperature in degrees Centigrade,
m = number of gallons per hour,
v = ,, revolutions per minute.
a, b, and c are constants for each separator.
b usually varies from 1·035 to 1·05.
c ,, ,, from 1·00 to 1·05.

c is appreciable chiefly with separators in which the adjustment of the thickness of the cream is made at the cream outlet, e.g. in the Alfa separator, in which c has the value 1·04 to 1·05.

Table 8.4 gives the results obtained with a separator for which the following formula was applicable—

$$f = 8{,}155 \times 1{\cdot}046^{(40-t)\cdot\frac{38}{t}} \times 1{\cdot}0471^{F} \times \frac{m^{\frac{2}{3}}}{v^{2}}.$$

Table 8.4

F	t	m	v	f	f calc.
Per cent	Degrees C	Gallons	Revolutions	Per cent	Per cent
15·5	32	350	5,600	0·05	0·04
42·0	32	350	5,600	0·12	0·13
51·0	32	350	5,600	0·175	0·194
52·6	32	350	5,600	0·210	0·207
56·3	32	350	5,600	0·247	0·246
65·0	32	350	5,600	0·330	0·369
60·4	38	350	5,600	0·22	0·228
62·1	38	350	5,600	0·25	0·247
51·0	32	240	5,600	0·14	0·14
53·0	27	350	5,600	0·30	0·27
42·0	32	325	5,200	0·15	0·14
70·0	76	120	5,600	0·07	0·07

The constant a depends on the following conditions—

(1) Size of drum and thickness of the layer of milk.
(2) The specific gravity of the milk serum and the fat.
(3) The unit in which the variables are expressed.

The first condition is that which can be varied by a difference in the type of separator.

The constant b depends chiefly on the viscosity (internal friction) of the milk serum; also, to a slight degree, on the cubical expansion of milk serum and milk fat, and on the friction of the liquid against the drum.

The constant c depends on the viscosity of cream and on the friction of the cream against the sides of the outlet.

It is naturally advantageous for a, b, and c to be as low as possible.

To obtain a low, the drum should be of large capacity and the formation of currents in the milk should be prevented; the discs placed inside the drum in separators of the Alfa type ensure the latter condition, and therefore decrease a.

To obtain b low, the exits, and especially the cream exit, should be as large as is compatible with the proper working of the separator; and the tubes, through which the skim-milk and cream leave the drum, as short and as straight as possible.

To obtain c low, cooling of the cream inside the drum should be avoided, and the cream exit large. Separators in which the adjustment of the thickness of cream is performed at the cream exit have a large c.

It is more difficult to express by a definite formula the amount of fat in skim-milk obtained by allowing milk to stand. Here we have not a definite space through which the globules of fat must pass, as in the cream separator, where the layer of milk is always of constant thickness; the space is determined by the depth of the layer of milk set.

It was pointed out by Golding, and the fact has been amply confirmed by Richmond, and later by Bolton and Revis, that the milk below the cream layer is of practically uniform composition, except at the extreme lower portion, as is shown by the following table. The milk contained 3·45 per cent fat, and the depth was 24 inches; samples representing a layer of about ½ inch were taken at various times and depths—

Table 8.5—Rising of cream in milk (percentages of fat)

	Percentage of fat						
Distance from top (in.):	24	21	18	15	12	9	6
½ hour	2·95	3·35	3·35	3·35	3·35	3·35	3·33
2 hours	2·40	2·87	2·90	2·95	3·02	3·02	3·05
4 ,, ...	2·17	2·52	2·55	2·57	2·57	2·57	2·67
8 ,, ...	1·60	2·30	2·35	2·37	2·37	2·37	2·35
24 ,, ...	0·20	1·67	1·80	1·82	1·87	1·87	1·90

Bolton and Revis have taken advantage of this fact to prepare a milk of any specified percentage of fat for infant feeding.

Thus, if milk of known fat content be allowed to stand, and the percentage of fat in the lower portion estimated, then the percentage of milk to be removed in the upper layer to give a milk of any specified percentage of fat is

$$\frac{100f-100f_1}{f_2-f_1}$$

where f = per cent fat in original milk,
f_1 = per cent fat in lower portions, and
f_2 = per cent fat desired in upper portion.

The formula—

$$v = \frac{s}{t} = \frac{br^{\frac{3}{2}}}{c}$$

may be transformed into—

$$t = \frac{k}{r^{\frac{2}{3}}}, \text{ where } k \text{ is a constant.}$$

Taking the diameter of the largest globules as 0·01 mm and the smallest as 0·0016 mm, we calculate that the smallest globules will take about fifty times as long to pass through a given space as the largest; Richmond deduces from his experiments that the largest fat globules move at the rate of 15 mm per hour. If we assume that the total weight of fat globules of any size is equal to the total weight of fat globules of any other size in an ordinary cream tube, we may expect roughly the following figures—

In 5 hours about 35 per cent of total fat will be found in the cream.
„ 10 „ „ 65 „ „ „ „ „
„ 24 „ „ 85 „ „ „ „ „

while from three to four days should elapse before virtually the whole of the fat is found in the cream.

From the equation

$$v = \frac{\sqrt{k \,.\, \frac{4}{3}\pi\,(d_s - d_f)}\, g \,.\, r^{\frac{3}{2}}}{c},$$

it will be readily seen that if the density of the fat varies, the time will be considerably affected. The density of solid fat at 60° F (15·5° C) is about 0·93; the density of liquid fat is about 0·92 at the same temperature; and, as has been shown by H. D. and S. O. Richmond, it is highly probable that the solidification of the fat is a process which takes time. The difference between the specific gravity of milk serum and milk fat is also accentuated at temperatures above 60° F; it is probable that when milk is cooled rapidly, the fat globules do not so easily attain the lower temperature as the serum. It would appear, theoretically, that there is a considerable advantage in setting milk for cream immediately after milking, and that the fat globules will rise at a much more rapid rate than if the milk be cooled and kept for some time. The experiments of Babcock substantiate this view completely; he finds that delaying the setting for even a short time affects materially the percentage of fat in the skim-milk.

A comprehensive mathematical treatment of the theory of the separation of cream has been given by Willsmann (1948). Sandelin (1949) has reported that addition of 74 mg per cent Ca to milk heated to 80° C caused a normal cream layer to form.

The globule membrane controls the milk emulsion and the rigidity of any gel resulting from the coagulation of the milk. The effect of emulsifying butter fat with various stabilising colloids has been studied by Tarassuk and Palmer (1939). Sharp and Krukovsky (1939b) have shown that the surface tension of cream and the separated milk vary according to the temperature of separation. The agglutinin responsible for clustering is differentially absorbed by liquid and solid fat, being concentrated on the fat globules at 5°—10° C and in the aqueous phase at 50° C. By using such skim milk for reconstitution a cream layer volume of 80–90 per cent can be obtained.

Dunkley and Sommer (1945) have reviewed the theories of cream rising and consider that the " agglutinin " is the main factor, although interfacial forces and protein hydration may be contributory factors. The agglutinin is absorbed by solid but not by liquid fat. It has been isolated and shown to be a euglobulin. Moyer (1940) finds that unwashed cream has an isoelectric point at pH 4·5 whereas that of washed cream is 3·75, thus offering suggestive evidence for a globule membrane.

Schwarz (1948) has shown by the electron microscope that the membrane consists of three layers, a middle layer of phosphatide droplets lying between a thin inner and a thick outer protein layer. Sandelin (1947) has suggested a double-layer theory in which the outer layer consists of a globulin-like protein and the inner of lecithin and related substances which are closely associated with the fat in the globule. He has shown that an artificial emulsion of fat and lecithin in water churns less readily as the lecithin and pH values increase. The fat-globule membrane portion has been shown to contain 15·6 per cent N, 0·6 per cent S, and 0·35 per cent P (ash-free basis). The ash ($Ca_2P_2O_7$) constituted about 3·5 per cent. This protein is so loosely held that on shaking some of it becomes detached, and it is of no significance in the stability of the fat emulsion. The lecithin is the important factor, and calcium ions easily destabilise the emulsion. Foaming assists in the churning process as the lecithin leaves the globules for the foam interfaces.

Jenness and Palmer (1949) report that washed cream contains about 0·6 g protein and 13 mg lipoid P per 100 g fat. The ratio of protein to phospholipid is generally about 2. Butter contains a fairly constant amount of protein (0·25 g per 100 g fat) but the phospholipoid is variable. Palmer (1944) has reviewed the theories of the " membrane " and shown that of all milk protein fractions, those from buttermilk and butter serum produce the greatest lowering of interfacial tension.

A comprehensive and critical discussion of the problems associated with the fat globule is presented by Mulder (1947a).

Some interesting differences in the rates of rise of fat globules in cows' and buffaloes' milk have been reported by Fahmi (1951). Refrigerator temperatures favoured the creaming of cows' milk but greatly retarded that of buffaloes' milk. With the latter a smaller cream layer and higher percentage of fat in the skim milk were obtained. At room temperature buffaloes' milk creamed very rapidly, probably due to the clumping of the fat globules.

Composition of skim-milk

Skim-milk differs solely from whole milk in the percentage of fat. In milk from which the cream has been removed by skimming, very wide variations are found in the percentage of fat; it varies from 0·4 per cent to over 2 per cent. Much lower percentages are found in separated milk, and the limits, 0·05 per cent to 0·3 per cent, are very rarely overstepped. By the removal of the fat the percentages of other solid constituents are increased slightly; this is caused by the constituents which were contained in 100 parts being left in about 96½ parts, by the removal of 3½ parts of fat.

The following is the average composition of well-prepared separated milk—

	Per cent
Water	90·48
Fat	0·12
Milk-sugar	4·88
Casein	3·22
Albumin	0·42
Ash	0·78

Control of separators

The most important point in the control of separators is the estimation of the fat left in the separated milk. A separator leaving a proportion of fat appreciably higher than that deduced from the formula given above is working badly, and the cause should be investigated at once. It is important that the speed be properly maintained, that the milk be at the right temperature, and that the exit tubes be not clogged up; the chemist should make a practice of visiting the separators daily while they are running and of checking the speed and temperature of the milk. At least one sample of separated milk should be tested from each " run " of the separator; these samples should be taken from the skim outflow tube at some period of the run, preferably not immediately after starting.

A further means of controlling the separators is to compare the total weight of the fat in the cream, the separated milk, and the milk left in the drum after separating, with the total weight of fat in the milk separated. This is done by weighing each product, multiplying the weight by the percentage of fat, and dividing by 100. The total weight of fat in the cream and separated milk should be nearly equal to that in the milk, the difference representing loss in separating; the average loss should not amount to more than 2 per cent of the total fat in the milk.

Separator slime

After running a separator, a viscous substance is found on the inside of the drum. It is usually of a dirty-white colour; but if the milk contains much solid impurity, as happens most frequently in the winter, it may be distinctly brown.

This by no means consists, as is often considered, of dirt and cow-dung, though it naturally contains these impurities if they are present in the milk. Microscopical examination shows it to contain—

(1) Inorganic impurities, i.e. dust gathered during transport, and earthy matter due to uncleanliness.

(2) Vegetable matters derived from the dust of the food given to the cattle, e.g. bark of hay, fine particles of cake, etc.; in many cases portions of leaves with stomata developed may be identified. Other portions of the vegetable matter have the cell walls considerably disintegrated; these have probably passed through the alimentary tract of the cow, and indicate the presence of cow-dung.

(3) Substances derived from the cow; hairs are often found; much epithelium from the udder of the cow, and possibly also from the hands of the milkers; and empty sacs (gland cells), which form a very large portion of the slime. (If cows are in ill-health, mucus, blood and pus may be present.)

Micro-organisms are very numerous; should the cows be afflicted with tuberculosis of the udder, *Myco. tuberculosis* may be found here.

The following composition is assigned to separator slime by Richmond and by Fleischmann, respectively—

Table 8.6—Composition of separator slime

	Richmond (Per cent)	Fleischmann (Per cent)	Richmond (Per cent) (hot)
Water	66·24	67·3	72·3
Fat	0·50	1·1	3·1
Casein (or analogous body)	22 (approx.)	25·9	18·1
Milk-sugar	0·5	2·1	4·0
Other organic matter	7·75		
Ash	3·01	3·6	2·5

It appears remarkable that when milk is separated at about 160° F the slime contains more water than when separated cold. The ash, however, has practically the same composition.

It is doubtful whether the substance returned as casein is wholly this body; it is probably a mixture of several proteins, including Storch's mucoid protein.

The following is the composition of the ash of separator slime—

	Per cent
Total ash	3·01
Soluble ash	0·166
Insoluble ash	2·844

consisting of

	Per cent
Silica	0·171
Iron oxide and alumina	0·012
Lime	0·654
Magnesia	0·225
Alkalis	0·559
Phosphoric anhydride	1·233

There are 0·675 equivalent of lime and 0·325 equivalent of magnesia to 1·506 equivalents of phosphoric anhydride, showing that the insoluble ash consists chiefly of (Ca, Mg) (Na, K)PO_4, like the insoluble ash of milk.

The quantity of separator slime averages about 0·032 part to 100 parts of milk separated, and varies within comparatively narrow limits—0·02 to 0·08—unless the milk be very dirty, when it may even reach 0·15; in a sample where the last figure was obtained, the slime was brown and very gritty.

Grimmer (1951) obtained separator slime to the extent of about 0·006 per cent of the milk used when fresh milk was separated at 3,000 r.p.m. The average analysis was 73·26 per cent water, 17·8 per cent nitrogenous substances, 3·34 per cent ether-soluble substances, 2·98 per cent ash and 2·62 per cent various other organic substances.

Clarification

Clarification, or the centrifugal removal of cells, dirt, etc., was formerly common in England for pasteurised milk and then almost entirely fell out of favour, though there are indications that it is once more being considered as a useful stage in processing. It can remove an amount of the order of 1 lb. of "separator slime" per 1,000 gallons of milk clarified. It has recently been shown that clarification has no effect on phosphatase content, albumin, renneting power and bacterial content, but affects the cream layer (Pedersen and Andersen 1947).

Technology

See the articles on "Cream" and "Creamline", and the article by Crossley on "Canned cream" in Davis's *Dictionary of dairying*.

CHAPTER 9

CONDENSED AND DRIED MILK

(1) CONDENSED MILK

Cows' milk contains, on the average, about 12·7 per cent of solid matter and about 87·3 per cent of water. It is obvious that if some method can be found for the removal of the whole or a portion of this water, the problem of transport would become very much easier; and if, further, the removal of the water leads to the possibility of the storage of the resulting product unchanged for an appreciable length of time, this will also tend to ease the difficulties of co-ordinating supply and demand.

Very early experiments, consisting of boiling off the water in pans open to the atmosphere, carried out in the eighteenth century, were not successful, and it appears that the first process which led to useful results was that for which Borden obtained a United States patent in 1849. In this process the milk was evaporated at a temperature considerably below its ordinary boiling-point by heating under reduced pressure. The process was so far successful that considerable quantities of this condensed product were used by the belligerents during the American Civil War. Modern processes may be said to date from this time.

Condensed milk apparently first became known in this country about 1856 (Pearmain and Moor, 1895) and some twenty years later Hehner (1879) could say: " . . . condensed milk being now very largely consumed not only as an addition to coffee, tea, etc., but principally as an exclusive food for infants."

In 1881 Voelcker (1881) made a report to the British Dairy Farmers' Association on the samples submitted in connection with a competition held by the Association. The majority of the samples submitted were those of partly-skimmed sweetened milks, although three consisted of unsweetened full-cream milks. It is interesting to note, as an indication of the progress in the methods of manufacture which has since been made, that Voelcker expressed the opinion that too much fat makes the product rancid, and that really good condensed milk is always made from skimmed milk or from milk unusually poor in cream.

Vieth (1883) stated that " Cows' milk has been condensed on a large scale for several decennaries and its use has spread over nearly all the countries of Europe."

The temperature which is used in the condensation of milk by the vacuum process is not sufficiently high to ensure that the product is sterile, and in any case it would be difficult, if not impossible, to carry out the subsequent processes of packing under perfectly aseptic conditions. Other steps have, therefore, to be taken to ensure that the product will remain unchanged for a reasonable length of time. This has been done in two ways: in one process by heating the containers, after hermetically sealing, to a temperature of some 240° F (116° C), and in another by adding ordinary sugar to the milk so that the finished product will contain some 40–45 per cent by weight of this substance. As the latter is, on the whole, a less difficult process, it was at first more widely adopted. The difficulties which were formerly found in the manufacture of the unsweetened variety have for the most part been surmounted, and large quantities of such milk are now on sale. In former years, unsweetened condensed milk was on sale as " Evaporated Milk ". This term was, however, at one time used to denote an unsweetened condensed milk which was not concentrated to the same degree as that now usual in this country. Such " evaporated milk " was imported from the U.S.A. prior to the introduction of the Condensed Milk Regulations in 1925.

The following are the minimum requirements for condensed and evaporated milk:

Description	*Minimum requirements* Milk fat per cent	*Minimum requirements* Total milk solids per cent
Full cream unsweetened	9·0	31·0
Full cream sweetened	9·0	31·0
Skimmed unsweetened		20·0
Skimmed sweetened		26·0

At present, however, under Reg. 60 CAA of the Defence General Regulations 1939, a lower standard is allowed for unsweetened full-cream imported condensed milk as follows—

Description	*Minimum requirements* Milk fat Per cent	*Minimum requirements* Total milk solids Per cent
Full cream unsweetened condensed milk (imported)	7·8	25·5

A concise introduction to the scientific study of condensed and evaporated milk has been written by Sykes in Davis's *Dictionary of dairying*. The standard textbook is Hunziker's *Condensed milk and milk powder*. See also Allen (1932), Scott (1932) and Savage and Hunwicke (1923).

Manufacture of condensed milk

(1) *Sweetened condensed milk*

The bulk milk, usually standardised with regard to its fat and solids-not-fat content so that the finished product will comply with the Regulations, is first heated for about 15 min. at about 175° F (79·5° C), although it seems probable that the ordinary temperature of pasteurisation—145° F (62·8° C) for thirty minutes—or superheating for a short time above the boiling point, gives a more stabilised product. The hot milk is then mixed with 15 or 16 lb. of sugar for every 100 lb. of liquid milk in one of several ways, probably the best being to dissolve the sugar by boiling in a small quantity of water and adding this solution to the liquid milk when the mixture is transferred to the vacuum pan. The condensing is carried out by heating the milk by means of metal coils brought to a temperature of about 235° F (113° C) by superheated steam. The process of condensation is usually a continuous one, fresh milk being drawn in whilst the proce of condensation is in operation. The progress of the condensation is controlle by drawing off small samples from cocks provided for the purpose and testin by appropriate rapid methods. Immediately after removal from the vacuu pan, the condensed product is rapidly cooled, it being kept in a state of continual agitation during the cooling process. It is then packed into containers.

(2) *Unsweetened condensed milk*

The preparation of unsweetened condensed milk is carried out in exactly the same way as for the sweetened variety as far as the completion of the condensing process except, of course, that no sugar is added. The condensed product, whilst still warm, is forced through very small nozzles under high pressure (the process termed " homogenisation ") in order that the fat globules may be broken up into others so much smaller that they will not subsequently rise to the surface and spoil the appearance of the finished article. After homogenising, the product is cooled and then packed into tins which are afterwards hermetically sealed. As previously stated, it is necessary for unsweetened condensed milk to be completely sterile, since there is no added sugar present to act as a means of preservation. This is carried out by heating the sealed cans to about 242·6° F (117° C) for some fifteen minutes, during which time they are kept in a state of continual agitation in order to break down the coagulum which is formed during the heating process.

Recent investigations

Eilers *et al.* (1947b) have found that evaporation does not lead to increase in the aggregation of protein particles but produces a lowering of pH due to changes in the ionic equilibrium of casein. Pre-pasteurisation increases the viscosity of the product. For evaporated milk a temperature of 95° C is desirable while for condensed 75° C is suitable. Tracy and Edman (1942) found that the use of enzyme-treated corn syrup lowered the pH of the condensed milk and led to browning. Corn syrup tended to produce age-thickening more readily than dextrose, and dextrose than sucrose. Skim milk was more prone to this fault than whole milk. High-temperature preheating gave greater stability and the separate heating of the milk and sugar solution was also advantageous. Hoskisson *et al.* (1944) have reported that replacement of half the sugar in skim condensed milk by dextrose or invert syrup increased the preservative power. Corn syrup caused thickening and invert syrup browning.

Webb *et al.* (1943) found that fore-warming at temperatures up to 150° C increased the heat-stability of evaporated milk, the higher temperatures usually being more effective than the normal commercial method of 95° C for 10 min. up to 25 per cent solids. Increasing solids decreased coagulation times. At a solids content of 32–37 per cent the two treatments became about equally effective. The best treatment appeared to be 120° C for 3 to 4 min., which permitted concentration up to 35 to 37 per cent solids, and the more rapid the heating the greater the stability. These workers confirmed the well-known instability of acid milk and the beneficial effect of phosphate. Later Deysher *et al.* (1944) studied viscosity aspects and found that viscosity increased rapidly just before coagulation, but that this point was very variable for different milks. These workers suggest that if a good " body " is required in milk processed at 115° for 20 min., the heat-stability value should not exceed 30 to 40 min. Increased " body " may be attained by increased solids content. Samples aged at 16° to 40° C all showed " thinning " initially and then settled down to a period of stability. Final thickening was very variable between samples but was most noticeable with milks of over 31 per cent solids. Storage below 21° C appeared to minimise loss of initial viscosity.

Webb and Bell (1943) have also compared various fore-warming treatments on *concentrated* milks after a normal fore-warming of the milk for 10 min. at 95° C. Heating the concentrated milk to 95° C for 10 or 20 min. resulted in a slight loss of heat stability. High temperature short-time heating improved stability, the maximum being obtained at 150° C with no holding. With separated milk, a zone of reduced heat-stability was obtained at 135°–145° C. The best heat-stability was obtained by fore-warming for 4 min. at 120° C and heating the concentrated milk momentarily to 150° C. The effect of this second heating on colour and flavour was only very slight. Bell *et al.* (1944) have found that milk fore-warmed at 65° C for 10 min. gave very low heat-stability after concentration, but gave better stability if sterilised for 135° C for 30 sec. Fore-warming for 4 min. at 120° C resulted in greater stability. Webb and Bell (1942) found that the relationship between stability and fore-warming temperatures varied for different milks. For each milk it was possible to determine a treatment which gave maximum stability, a stability greater than that obtainable by addition of stabilising salts. Skim-milk is invariably more stable than the original whole milk.

Webb and Hufnagel (1948) find that the viscosity of sweetened condensed milk increases logarithmically with temperature and arithmetically with time. The theoretical freezing point is about 5° F (—15° C). Nelson (1949) has studied the effect of different conditions of heating on various physical properties of evaporated milk. Increasing the times of heating increased viscosity and surface tension together and decreased reflectance. Figures are given showing the changes in these and also the extent of grain formation for various combinations of time and temperature. Data for increases in viscosity of stored condensed milk when held at 60° F (15·5° C) for 124 days have been given as follows (Anon, 1949b)—

Preheating	*Viscosity change in poises*
180° F for 10 min.	360 to 1,100
180° F for 10 min. plus 240° F for 30 sec.	220 to 534
180° F for 30 min. plus 240° F for 30 sec.	50 to 457
240° F for 30 sec.	20 to 264

Stanworth (1944) considers that a modified Gerber test is sufficiently accurate for control purposes. If the s.n.f. exceeds 2·44 times the fat, over-concentration is necessary to attain the 9 per cent fat requirement. If sugar to 1·4 times the total milk solids is added, some crystallisation may occur on cooling. It is therefore necessary to add cream, and this very useful paper gives data for amounts of cream to be added and also for Baumé hydrometer readings.

Deysher and Webb (1948) have shown that the crystals deposited in evaporated milk consist usually of about 98 per cent tricalcium citrate with varying amounts of tricalcium and trimagnesium phosphates. Zimmerman and Oberg (1948) have found that evaporated milk yielded, per litre of reconstituted milk, 134 and 70 mg of volatile acid (as formic) and 32 and 40 mg of formic acid by steam and vacuum distillation respectively.

The composition of condensed milk

The composition of condensed milk must be considered under three main headings: (1) sweetened whole milk, (2) sweetened skimmed milk, which may be prepared from milk deprived of a part or practically the whole of its fat, and (3) unsweetened whole milk. There is a fourth possibility, namely, unsweetened skimmed milk, but this is not produced in any material quantity.

(1) *and* (2) *Sweetened whole milk and skimmed milk*

Hehner (1879) quotes an average of four analyses by Hassal and some figures of Wanklyn, which are given below—

Table 9.1—Analyses of condensed milks, 1879

	Hassal	Wanklyn
Water	25·7	20·5
Casein	16·8	11·0
Lactose	15·4	56·1
Sucrose	29·0	
Fat	10·3	10·4
Ash	2·8	2·0
	100·0	100·0

Hehner gives the results of thirteen analyses, all of sweetened milks, which he had conducted himself, and states that they " appear to be totally and absolutely unfit to be used as a substitute for mother's milk "; he also states that certain aspects of the labelling were objectionable.

Voelcker (1881) gives an analysis of three samples of unsweetened milk which contained 17·1 to 14·3 per cent of fat and 24·0 to 27·0 per cent of solids-not-fat. A number of sweetened milks contained from 6·2 to 9·9 per cent of fat. Voelcker expresses the opinion that such products are preferable to milk powders obtained by evaporating skimmed milk completely to dryness with the addition of cane sugar, and reducing the residue to powder.

In 1898 a Metropolitan magistrate convicted a defendant for the sale of a skimmed condensed milk, reported as being a " machine-skimmed milk ", which had had 97 per cent of the original fat abstracted. The case was taken to appeal before a divisional court, but the appeal was dismissed.

Vieth (1883) states that condensed mares' milk was first heard of in 1882, when an English company established a factory for this at Samara in the steppes of south-eastern Russia. Two samples were examined—

Table 9.2—Composition of condensed mares' milk

	Sample A	Sample B
Water	17·9	18·8
Fat	12·1	10·1
Protein	13·5	15·2
Sugar	54·9	54·1
Ash	1·6	1·8

An editorial in *The Analyst* of 1883 (**8**, 171) states that 0·2 of a grain of borax per gallon was added to the unconcentrated milk in the preparation of unsweetened condensed milk at the factory of the First Swiss Alpine Milk Co. An analysis due to Fresenius was—

Table 9.3—Composition of unsweetened condensed milk, 1883

Casein	10·6
Albumin	1·3
Fat	10·9
Lactose	14·2
Borax	0·6
Other mineral matter	1·8
Water	60·6
	100·0

It was stated that the directors attributed the fact that the colour was much darker than that of ordinary milk to the larger quantity of green food given to the cows, but the editorial says: " The dark chocolate colour of most of the unsweetened condensed milks is much more likely to be due to slight decomposition of either the milk-sugar or casein, caused by the high temperature employed in sterilisation."

An editorial in *The Analyst* of 1884 (**9**, 34) drew attention to successful prosecutions in Liverpool for the sale of condensed milks deficient in fat.

Faber (1889) describes a machine-skimmed condensed milk containing as little as 0·53 per cent of fat. This writer states that it was said at this time that unsweetened condensed milk was diluted and added to fresh milk for sale as such, and that a society of dairy farmers had expressed a wish that this practice should be stopped and had looked for a method of detecting the mixture. Richmond and Boseley (1893) gave methods of analysis and expressed the opinion that the words " skimmed " and " separated ", when applied to milk, were not synonymous.

Pearmain and Moor (1895) published the results obtained on the analysis of 50 different brands. Of these, six were unsweetened, containing from 9·5 to 12·4 per cent of fat and 29·9 to 44·6 per cent of total solids. Of the 44 samples of sweetened condensed milk, 13 were prepared from separated milk, two from partially separated milk, and 15 from whole milk. This paper refers to a report by Dyer (1895) who determined the amount of fat in seventeen samples of condensed milk, all except one of which had been prepared from separated or skimmed milk.

Allen (1895) gives results for the examination of 29 samples of which three were unsweetened and 26 sweetened, one in each class being a duplicate. The unsweetened samples contained 8·8, 11·1 and 10·4 per cent of fat respectively, and 28·4, 35·2 and 36·1 per cent of solids-not-fat. In the sweetened samples, the total solids varied from 79·1 to 66·3 per cent and the fat from 13·5 to 0·2 per cent.

Coutts (1911) collected some of the above results and others due to Lloyd, McCrae, Dodd and Garratt, the Government Laboratory, and Monier-Williams. These results are summarised in the following tables—

Table 9.4—Sweetened condensed milk (full cream), 1895 to 1910

Brand	No. of samples	Total solids	Fat	Cane sugar	Date
A. Swiss	20	70·4 to 77·4	0·1 to 13·7	37·2 to 41·5	1895 to 1910
B. ,,	3	73·6 ,, 76·0	10·2 ,, 11·8	—	1895 ,, 1907
C. Dutch	2	76·3 & 77·7	10·4	—	1908 & 1910
D. ,,	4	77·3 to 83·6	8·9 to 10·5	—	1907 to 1910
E. ,,	8	70·6 ,, 77·2	5·6 ,, 10·5	40·5	1895 ,, 1908
F. Norway	8	72·5 ,, 77·2	8·1 ,, 13·0	37·3 to 41·4	1895 ,, 1910
G. Dutch	2	75·0	9·7 & 10·1	—	1907 & 1909
H. Swiss	10	73·6 to 76·7	9·1 to 11·1	38·7 to 41·0	1895 to 1910
I. Dutch	2	68·1	9·8 & 11·1	—	1895 & 1909
J. French	4	76·6 to 76·9	9·0 to 9·8	39·2 to 44·6	1909 to 1910
K. Dutch	1	75·9	10·4	—	1909
L. ,,	1	78·3	10·3	—	1909
M. U.S.A.	1	73·4	10·8	39·2	1910
N. Dutch	5	73·4 to 76·7	11·0	43·4	1910
O. ,,	1	76·8	11·0	43·4	1910
P. ,,	3	72·4 to 76·6	10·3 to 12·4	36·1 to 39·8	1895 to 1910

Table 9.5—Sweetened condensed milk (machine-skimmed), 1895 to 1910

Brand	No. of samples	Total solids	Fat	Cane sugar	Date
A. Dutch	6	67·4 to 75·5	0·2 to 1·5	44·3	1895 to 1910
B. ,,	3	69·4 ,, 71·3	0·9 ,, 1·3	43·9	1895
C. ,,	5	71·0 ,, 79·1	0·6 ,, 4·3	45·9 to 52·7	1895 to 1910
D. ,,	6	72·5 ,, 74·9	1·0 ,, 6·5	45·8 & 49·0	,,
E. ,,	4	74·0 ,, 75·0	0·8 ,, 1·3	39·5 to 44·7	,,
F. ,,	4	66·6 ,, 71·4	0·1 ,, 2·0	43·5	,,
G. Gt. Britain	5	56·9 ,, 74·5	0·4 ,, 4·2	30·4 to 50·4	,,
H. ,,	2	75·9 & 77·1	0·9 & 1·2	38·5	1909 & 1910
I. French	1	71·6	1·5	44·9	1910
J. Dutch	1	73·2	0·6	40·8	1909
K. Saxony	1	75·4	0·6	46·1	1910

From the above tables it will be seen that sweetened condensed milk, up to about the year 1910, contained on the average a little more than 40 per cent of cane sugar, the total solids being, at least in the better brands, just over 70 per cent, while the fat was usually about 10 per cent. It will be further observed that the Condensed Milk Regulations are based on about the average of the better samples. Since the Regulations came into force, the somewhat wide fluctuations which were formerly observed are no longer found. True it is that the richer milks no longer occur, but it is now very unusual to find condensed milks much below the standards of the regulations, and a stabilised product can now be obtained. The change in the composition of sweetened condensed milk is well shown by the analyses which were made in the Lancashire County Laboratory during the years 1908 to 1934. These are set out in the following table—

Table 9.6—County of Lancaster: composition of sweetened whole condensed milks, 1908 to 1934

Total solids per cent	No. of samples*		Fat per cent	No. of samples	
	1908–1923	1924–1934		1908–1923	1924–1934
70·0 to 70·9 ..	5	0	7·0 to 7·9 ..	5	0
71·0 ,, 71·9 ..	4	0	8·0 ,, 8·9 ..	8	2
72·0 ,, 72·9 ..	6	3	9·0 ,, 9·1 ..	3	2
73·0 ,, 73·9 ..	13	18	9·2 ,, 9·3 ..	3	1
74·0 ,, 74·9 ..	7	19	9·4 ,, 9·5 ..	2	3
75·0 ,, 75·9 ..	6	9	9·6 ,, 9·7 ..	3	1
76·0 ,, 76·9 ..	3	2	9·8 ,, 9·9 ..	4	13
77·0 ,, 77·9 ..	5	0	10·0 ,, 10·4 ..	16	9
78·0 ,, 78·9 ..	2	0	10·5 ,, 11·2 ..	8	0

* One sample contained 64·1 per cent.

In the following table will be found the results obtained in the Lancashire County Laboratory for a number of samples of sweetened machine-skimmed condensed milk during the years 1904 to 1934—

Table 9.7—County of Lancaster: composition of sweetened machine-skimmed condensed milks, 1904 to 1934

Total solids Per cent	No. of samples	Fat per cent	No. of samples
66·0 to 66·9	2	0·00 to 0·09 ..	4
67·0 ,, 67·9	4	0·20 ,, 0·29 ..	2
68·0 ,, 68·9	1	0·30 ,, 0·39 ..	8
69·0 ,, 69·9	2	0·40 ,, 0·49 ..	11
70·0 ,, 70·9	10	0·50 ,, 0·59 ..	5
71·0 ,, 71·9	22	0·60 ,, 0·69 ..	4
72·0 ,, 72·9	35	0·70 ,, 0·79 ..	0
73·0 ,, 73·9	31	0·80 ,, 0·89 ..	0
74·0 ,, 74·9	5	0·90 ,, 0·99 ..	1
75·0 ,, 75·9	2	1·00 ,, 1·09 ..	1
Totals	114	Totals	36

Richmond reported the results of a large number of analyses of condensed milks manufactured before the Regulations came into force. These results are summarised in Tables 9.8 and 9.9, from which obviously unsatisfactory samples have been omitted.

Table 9.8—Percentage composition of sweetened condensed whole milk

	Average	Max.	Min.
Water	25·30	31·9	16·4
Fat	10·59	13·9	7·5
Milk-sugar	13·89	17·6	11·6
Cane sugar	38·93	46·2	32·4
Proteins	9·27	12·3	7·3
Ash	1·96	2·4	1·6

Table 9.9—Percentage composition of sweetened condensed skimmed milk

	Average	Max.	Min.
Water	28·98	38·8	20·9
Fat	0·67	2·2	0·1
Milk-sugar	14·94	17·0	10·9
Cane sugar	42·45	50·4	30·4
Proteins	10·41	12·3	7·6
Ash	2·26	2·9	1·6

The sweetened condensed milks of commerce now usually have a composition little removed from the averages given in Table 9.10—

Table 9.10—Average composition of sweetened condensed milks

Constituent	Whole milk per cent	Skimmed milk per cent
Fat	9·9	0·5
Total solids	73·5	72·5
Ash	2·1	2·3
Proteins	9·3	10·2
Lactose	13·8	15·2
Cane sugar	38·0	43·8
Other constituents	0·4	0·5
Water	26·5	27·5

Recent (1950) analyses from the Government laboratory are given in Table 9.11 (constituents per cent)—

Table 9.11

(*a*) *Full cream sweetened*

Number of samples considered: 100

	Average	*Max.*	*Min.*
Sugar	41·97	44·3	40·2
Total milk solids	32·50	35·0	30·3
Fat	9·36	10·4	0·2
Acidity	0·28	0·4	0·2

(*b*) *Machine skimmed sweetened*

Number of samples considered: 100

Sugar	45·16	47·10	42·6
Total milk solids	26·74	29·18	24·5
Acidity	0·30	0·35	0·25

(3) *Unsweetened milk*

The composition of unsweetened condensed milk is similar to that of sweetened, except of course that no cane sugar is present, and therefore there is much less total-solid matter and more water. The following results were obtained in the Lancashire County Laboratory during the years 1917 to 1934. It will be noticed what a considerable improvement took place subsequent to 1923 as a result of the Regulations.

Table 9.12—County of Lancaster: composition of unsweetened condensed milks, 1917 to 1934

Total solids per cent	No. of samples		Fat per cent	No. of samples	
	1917–1923	1924–1934		1917–1923	1924–1934
16·5 to 20·0	2	0	4·2	1	0
25·0 ,, 25·9	12	0	6·0 to 6·9	2	0
26·0 ,, 26·9	5	0	7·0 ,, 7·9	11	0
27·0 ,, 27·9	3	0	8·0 ,, 8·4	6	0
28·0 ,, 28·9	1	0	8·5 ,, 8·9	3	1
29·0 ,, 29·9	1	0	9·0 ,, 9·1	0	6
30·0 ,, 30·9	0	4	9·2 ,, 9·3	1	16
31·0 ,, 31·9	0	51	9·4 ,, 9·5	0	9
32·0 ,, 32·9	0	10	9·6 ,, 9·7	0	0
33·0 ,, 33·4	0	1	9·8 ,, 10·0	0	3
TOTALS	24	66	TOTALS	24	35

Richmond gives the following figures—

Table 9.13—Percentage composition of unsweetened condensed milks
(*Richmond*)

	Average	Max.	Min.
Water	63·73	68·9	60·6
Fat	10·80	12·5	8·8
Milk-sugar	13·99	16·0	9·1
Proteins	9·36	10·3	8·0
Ash	2·06	2·3	1·5

The following remarks due to Richmond are sufficiently interesting to reprint; they should, however, be interpreted in conjunction with the results obtained by modern methods of analysis—

> " It is noticed that the totals of analyses of condensed milk almost invariably add up distinctly below 100 per cent; it is probable that the milk-sugar is underestimated. In condensed milk the layer of solution which is attracted round the fat globules by surface energy has probably a composition which is identical with the composition of the liquid in which the globules are suspended. When condensed milk is diluted with water, it is doubtful whether the liquid in this layer is diluted by the water, as it is held by great force, and acts as though separated by a semi-permeable membrane, through which the dissolved solids must pass by osmosis. As the milk is usually diluted with cold water, this process of osmosis takes a considerable time, and the whole of the milk-sugar is not obtained in solution, but a portion is taken down by the fat globules, when they are removed previous to the estimation of the milk-sugar. The same cause can be assigned to the fact that the fat globules in diluted condensed milk rise with such extreme slowness; a dense layer round the globules increases their mean density, and makes this approach nearly to the density of the serum."

The unsweetened milks of commerce now usually have a composition similar to that given in Table 9.14—

Table 9.14—Composition of unsweetened condensed milks

	Per cent
Fat	9·3
Total solids	31·6
Ash	1·8
Proteins..	8·2
Lactose	12·0
Other constituents	0·3
Water	68·4

The following results were obtained in the Government laboratory in 1950 (constituents per cent)—

Table 9.15

Full cream unsweetened

(1) *Complying with the standard of the Public Health Condensed Milk Regulations*—64 samples. These were made in Great Britain, Northern Ireland and other European countries.

Class 1—number of samples: 64

	Average	*Max.*	*Min.*
Total milk solids	31·75	32·9	30·7
Fat	9·64	10·3	9·0
Acidity	0·31	0·45	0·2

(2) *Complying with the standard allowed by the Defence Regulations*—36 samples from New Zealand.

Class 2—number of samples: 36

	Average	*Max.*	*Min.*
Total milk solids	27·10	27·6	26·2
Fat	8·49	9·2	7·8
Acidity	0·29	0·45	0·2

This " acidity " is the acidity titratable to phenolphthalein expressed as lactic acid.

(2) MILK POWDER OR DRIED MILK

The obvious advantages attaching to condensed milk, namely, ease of carriage and preservation of an otherwise easily perishable article, could be carried to a still greater extent by removing the whole of the water and obtaining the milk as a dry powder. Such powders were known before 1900, but most, if not all, of them seem to have contained quantities of added sugar of the order of 40 per cent.

In 1901 Campbell's process for the preparation of a dried milk was patented in England. In this process the milk is first highly concentrated by blowing heated air through the liquid, after which it is run into a rotating drum and further dried by hot air until it becomes brittle, when it is broken up into small pieces, sieved, completely dried by a current of hot air in a canvas drum, and then ground into powder. Although this process, with later modifications, has been used to a certain extent, the bulk of the dried milk at present on the market is prepared by several processes which may roughly be divided into two classes, viz. *roller* and *spray* processes respectively.

In the roller process, milk, either in its original state or after condensation in some form of vacuum pan, is allowed to run in a thin film on to steam-heated metal rollers which revolve at about 16 revolutions per minute. The rollers are heated internally by means of superheated steam, the temperature of which is of the order of 146° C. The milk becomes dry before a revolution is completed, when the dried film is removed by means of a metal scraper blade placed at an angle to the surface of the roller. The dried product is afterwards broken up and sieved.

In the spray process, the milk, usually pre-condensed, is forced by means of a pressure pump through a fine orifice in the side of a large drying chamber, whilst at the same time heated air is pumped into the drying chamber through a series of small inlets surrounding the spray nozzle. The milk, being in a very finely divided state, is dried almost immediately and falls as a dry powder on to the floor of the chamber.

The best spray-dried powder contains rather less moisture than roller-dried milk and has a greater solubility. A good spray-dried powder has a solubility in cold water of nearly 100 per cent, whilst roller-dried milk does not usually have a solubility under similar conditions exceeding 85 per cent.

An informative article on the technical aspects of milk powder (also called dried milk) has been written by Sykes in Davis's *Dictionary of dairying*. The standard textbook is Hunziker's *Condensed Milk and Milk Powder*.

A review of milk powder methods in Germany has been given by Schober (1938). This is specially useful for the technological and costing aspects of the subject. Technical details are also given by Allen (1932) and Scott (1932). For infant feeding aspects, see Coutts (1911).

The composition of dried milk

It is generally agreed that ordinary cows' milk has, on the average, a composition approaching—

Table 9.16—Average composition of milk

Fat	3·75
Proteins	3·40
Milk-sugar	4·70
Mineral matter	0·75
Other ingredients	0·05
Water	87·35
	100·00

If such an average milk were dried until it contained about 3 per cent of water (the whole of the water is not removed in preparing " dried milk " on the manufacturing scale), the finished product would have the following composition—

Table 9.17—Calculated composition of dried milk

Fat	28·7
Proteins	26·0
Milk-sugar	36·0
Mineral matter	5·8
Other ingredients	0·5
Water	3·0
	100·00

This may be regarded as the composition of a dried milk prepared from a whole milk of average quality. Other dried milks are, however, prepared in practice in which varying proportions of fat are removed before the drying process is carried out, whilst in these and in other cases lactose may be added.

The following table contains some analyses of dried milks carried out in the Lancashire County Laboratory—

Table 9.18—Percentage composition of dried milk, 1933

Sample	Water	Fat	Proteins	Ash
1	4·3	23·2	25·4	6·1
2	6·2	22·6	26·1	6·1
3	4·3	23·8	24·5	6·2
4	3·9	25·2	24·2	6·2
5	5·5	13·8*	26·3	7·2
6	5·0	25·4	24·8	5·9
7	6·4	0·7†	30·7	7·6
8	2·4	19·7‡	18·1	3·8
9	6·4	28·6	23·9	5·3
10	2·5	26·1	26·9	6·0
11	5·6	26·0	24·5	5·6
12	6·7	26·0	25·1	5·8
13	5·3	14·6‡	18·2	4·4

* Half-cream. † Machine-skimmed. ‡ Contained added lactose.

Richmond gives the following analyses—

Table 9.19—Percentage composition of milk powders (*Richmond*)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Moisture ..	6·39	4·92	3·30	3·55	4·74	5·15	6·03
Fat	27·35	27·98	23·97	2·55	29·16	19·90	25·60
Milk-sugar ..	31·42	34·16	37·32	45·60	32·24	34·96	32·83
Cane sugar ..	—	1·25	1·53	2·80	—	—	2·00
Protein ..	27·48	24·59	26·38	35·45	26·66	31·10	23·84
Ash	6·00	6·24	6·19	7·89	5·63	7·11	6·44
TOTAL ..	98·64	99·14	98·69	97·84	98·43	98·22	96·74
Water of hydration	1·65	1·80	1·96	2·40	1·70	1·84	1·73
TOTAL ..	100·29	100·94	100·65	100·24	100·13	100·06	98·47
Change of temperature on mixing with water	—0·2°	0·0°	—0·2°	—0·4°	—0·2°	—	—0·3°

Note—In these samples the proteins were precipitated by mercuric nitrate, and the milk-sugar is probably underestimated by 0·5 to 0·8 per cent.

It is noticed that none of the analyses adds up to 100 per cent; the milk-sugar has been calculated as anhydrous sugar, and here lies the reason for the deficiency.

On shaking with water the solid residue obtained by drying milk on the water-bath, in which the milk-sugar partially exists as anhydrous sugar, a rise of temperature always takes place; anhydrous milk-sugar mixed with water always causes a rise of temperature, whilst hydrated milk-sugar causes a fall of 0·55° if excess is added. The milk powders examined, with one exception (No. 2), all caused a fall of temperature, and it is seen that the addition of the water of hydration to the total gives figures which are but slightly in excess of 100 per cent; both the change of temperature and the slight excess over 100 per cent indicate that the bulk of the milk-sugar, though not all, exists as hydrated sugar. Sample (2) differed in appearance from the others, being a heavy powder instead of light and flaky, and had doubtless been more dried and probably contained a considerable proportion of anhydrous sugar; it was noticed that the addition of the water of hydration would make the total nearly 101 per cent. Sample (7) gave a low total, which was probably accounted for by the presence of invert sugar.

It will be noticed that samples (2), (3), (4) and (7) contained small quantities of cane sugar; that in sample (2) was admittedly added in the form of saccharate of lime; it was certainly so added, judging from the analytical figures, in (7).

In Table 9.20 the composition of the original milks, on the assumption that they contain 9·0 per cent of solids-not-fat, is given—

Table 9.20—Percentage composition of original milks (*Table* 9.19)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Fat	3·79	3·88	3·09	0·26	4·07	2·45	3·65
Milk-sugar ..	4·36	4·73	4·87	4·62	4·50	4·30	4·68
Protein ..	3·81	3·41	3·40	3·58	3·71	3·82	3·40
Ash	0·83	0·87	0·80	0·80	0·79	0·87	0·92
CaO	0·19	0·21	0·17	0·17	0·17	0·19	0·27
P_2O_5	0·23	0·24	0·23	0·23	0·23	0·29	0·24
Acidity ..	8·4°	13·2°	16·8°	16·5°	19·6°	—	11·4°

From the table it is seen that No. 4 was made from separated milk, and No. 6 from milk deprived of a portion of its cream. The milk used to prepare No. 3 was only just above the Government limit. The normal percentages of lime and phosphoric anhydride in milk are 0·17 per cent and 0·23 per cent respectively, but vary somewhat with the protein, and the normal acidity is not far from 20°. From a consideration of the results, it appears that Nos. 2 and 7 received an addition of saccharate of lime, and No. 6 received an addition of a phosphate. Nos. 3 and 4 contained cane sugar, but there is no evidence of the addition of saccharate of lime. No. 1 probably received an addition of sodium carbonate, as the lime is not high enough, considering the high protein content, to indicate an addition of this substance, and No. 5 appears to have received no addition whatever.

Table 9.21—Percentage composition of dried milk powders

	Water	Fat	Protein	Milk-sugar	Ash	NaCl	CaO	P_2O_5
WHOLE-MILK POWDERS								
Av.	4·12	26·90	24·70	37·09	6·04	1·17	1·35	1·64
Min.	1·85	22·58	22·88	35·13	5·44	0·92	1·23	1·46
Max.	5·75	31·28	27·75	41·39	7·58	1·59	1·75	1·77
PARTIALLY SKIMMED POWDERS								
Av.	5·71	15·44	28·79	43·18	6·79	1·29	1·57	1·82
Min.	5·00	11·74	28·18	42·29	6·60	1·15	1·51	1·73
Max.	6·50	18·25	29·76	44·82	7·10	1·50	1·64	1·98
SKIMMED POWDERS								
Av.	6·25	1·41	32·81	49·84	8·04	1·67	1·75	2·14
Min.	2·29	0·67	31·09	45·65	7·00	1·21	1·42	1·71
Max.	9·18	4·73	37·23	52·58	9·66	2·25	1·92	2·44

	Water	Fat	Protein	Milk-sugar	Cane sugar	Ash	NaCl	CaO	P_2O_5
WHOLE-MILK POWDERS CONTAINING CANE SUGAR									
Av.	5·15	25·36	23·48	36·52	2·14	6·26	1·19	1·50	1·62
Min.	3·56	22·65	22·27	34·77	0·35	5·70	0·86	1·29	1·55
Max.	6·10	28·67	25·37	38·83	2·94	6·75	1·51	1·70	1·69
PARTIALLY SKIMMED POWDERS CONTAINING CANE SUGAR									
Av.	5·86	13·51	27·85	43·16	2·15	6·80	1·41	1·56	1·82
Min.	5·40	7·15	25·01	39·28	0·77	6·40	1·15	1·51	1·61
Max.	6·17	21·92	29·63	46·32	3·85	7·10	1·62	1·62	1·93
SKIMMED POWDERS CONTAINING CANE SUGAR									
Av.	6·11	1·55	33·21	48·73	0·82	8·55	1·81	1·81	2·11
Min.	4·08	0·70	30·69	45·84	0·20	7·10	1·30	1·64	1·89
Max.	10·87	2·25	35·57	52·58	1·68	9·56	2·34	2·20	2·21

At the Government Laboratory a number of samples of dried milk have been examined (Table 9.21); in these the milk-sugar has been calculated as hydrated sugar, as they confirm Richmond's observation that there is a fall of temperature in practically all cases on dissolving the milks in water. In four samples, evidence of the addition of sodium carbonate was found, and six samples show clear evidence of the addition of lime, while in four cases it is probable that sodium phosphate was used. The fat was found to be normal in all cases except one sample of Russian origin which showed a low Reichert-Wollny figure.

Milk powders containing considerable quantities of cane sugar are also made. Two samples examined by Richmond and one by the Government Laboratory had the following compositions—

Table 9.22—Cane sugar in milk powders

	Richmond		*Govt. Lab.*
	Per cent	*Per cent*	*Per cent*
Fat	15·2	13·5	17·94
Milk-sugar	21·7	21·3	21·89
Cane sugar	42·5	40·9	39·73
Protein	15·1	14·9	13·05
Ash	3·3	3·2	3·59

These had a slightly rancid odour and taste. When dissolved in water some of the fat was not emulsified.

In 1950 a random group of 100 samples of full-cream roller-dried milk received in the Government laboratory gave (per cent)—

		Average	*Max.*	*Min.*
	Fat	28·05	29·3	26·3
A group of 30 half-cream roller-dried milks gave:	Fat	17·0	18·0	15·2
A group of 18 samples of dried buttermilk gave:	Fat	5·95	7·9	4·5

The solubility of full-cream roller-dried milk as determined by Parsons' (1949) method is partly dependent upon the degree of neutralisation to which the milk was submitted. Samples can be classified as follows—

Group 1—Solubility 90–95 per cent. These are usually good-quality powders but have been neutralised to a considerable extent.

Group 2—Solubility 85–90 per cent. These are usually powders of good quality either not neutralised or neutralised to a less extent than the powders in Group 1.

Group 3—Solubility 80–85 per cent. These are probably not neutralised. The powder may be of good quality or may show signs of inferior technique in manufacture.

Group 4—Solubility below 80 per cent. Solubilities of this order are poor. They may be the result of unsatisfactory methods of manufacture and may also arise from long storage, especially if the powder is allowed to absorb moisture.

Density of dried milk powders

Lea and Gane (1946) find that the true density of 27 per cent fat powder is 1·27, but for the purposes of calculating enclosed air, a value of 1·33 should be used. Ault (1948) has also described a method for determining the apparent density of milk powder. Stamberg and Bailey (1940) have evolved a "density index" method for milk powders. Values varied from 24 to 125 for spray-dried powders. Roller and well-ground spray powders had densities of 1·47 and 1·46. Jack (1939a) has obtained contraction values of 27–36 cu mm per g for roller and 51–82 for spray powders, the change usually being complete in 22 hr. for spray and 28 hr. for roller powders. He further finds (1939b) that the maximum volume contractions on water sorption were 43–58 for roller and 69–102 for spray powders (cu mm per g), the sorptions being 15–19 and 24–36. A later paper (1940) gives water sorption figures for roller and spray powders. The values vary considerably with the method used.

Webb and Hufnagel (1943) have used pressures of 200 to 3,200 lb. per sq. in. to make blocks of spray-dried skim- and whole milk powder, the densities of 0·60 and 0·55 being increased to from 0·78 to 0·97 and 0·73 to 1·01 respectively. However as rapid compression did not allow for air escape, some 5 to 15 per cent of the volume decrease was recovered on releasing the pressure. The paraffin-lined tight oak barrel was the best container; the rectangular fibre box lacked strength and was not moisture-proof.

Manus and Ashworth (1948a) have shown that pre-condensing to a solids content of 40 per cent results in a higher solubility of spray-dried powder than when pre-condensed to 20 per cent. After 6 months' storage at 45° F (7·2° C) all powders were completely soluble. These authors have also described a method for determining powder density and have found that density and ease of reconstitution increase with increasing pre-concentration.

Factors affecting keeping quality of milk powders

(i) *General*

Thiel and Pont (1945) failed to find any correlation between keeping quality and moisture, Fe, Cu, and bacterial count for spray and roller powders. Thiel (1945) also found that compressed blocks protected by wax and/or cellophane did not keep as well as the gas-packed powder. Bryce and Pearce (1946) have published data on the effect of storage temperature for spray powders. "Storage flavours" developed at 80° F (26·7° C) and higher but not at 40° F (4·5° C) and 60° F (15·6° C). Greenbank *et al.* (1946) suggest that the rate of peroxide formation is a good index of keeping quality. They report a temperature coefficient of 1·6 per 10° C. Dahle and Josephson (1943) have shown that milk powders with the lowest lecithin content have the best keeping quality. Shtalberg (1950) finds the best anti-oxidants for dried milk products to be wheat and maize oils, and vitamin E and ascorbic acid together.

Jenness *et al.* (1946) have fractionated the fluorescing substances in milk powder and find that half of that in the acetone–water fraction is riboflavin, the remainder being proteins; that in the acetone–ether fraction is lipid in nature, while proteins contributed to the fluorescence in the KCl extract. The fluorescence of the first and third fractions increased on storage and appeared to be due to the proteins and lactose. This phenomenon was related to the production of stale flavour. Crowe *et al.* (1946) have found that storage at

60° C increases the amount of reducing materials. This may be related to the destruction of ascorbic acid. Coulter *et al.* (1948) observed that during the storage of milk powder the lactose decreased and acidity increased, carbon dioxide was produced and oxygen absorbed, substances reducing indophenol and ferricyanide were produced, extractable fluorescent substances were formed, browning occurred and a stale or " burnt feather " flavour developed.

Decker and Ashworth (1951) have further shown that ascorbic acid at 0·01 per cent and sodium citrate at 0·1 per cent per litre of reconstituted milk do not significantly prolong the life of air-packed powder at 85° F (29·5 C). Sodium citrate has a slightly synergistic effect on the ascorbic acid, increasing the storage life from about three months to five months longer than when ascorbic acid is used alone. Staleness rather than tallowiness appeared in these samples. The degree of off-flavour developed could be correlated with the E_h value developed in the powder.

Hillig (1940) has shown that lactic acid disappears from skim-milk powders during storage.

(ii) *Moisture*

Working with skim-milk powder Henry *et al.* (1948) found little change in powders of 3 and 5 per cent moisture under various storage conditions. Powders of 7·6 per cent moisture rapidly became insoluble, discoloured and unpalatable, absorbed oxygen and produced CO_2 and suffered a fall in pH, free amino-N and soluble lactose. In addition there was an increase in the amount of sugar linked with protein and in the ferricyanide-reducing power of the powder. These changes were much more marked at 37° than at 20° and 28·5° C and appeared to be largely concerned with a reaction between the ϵ-amino groups of the lysine residues and the aldehyde group of the reducing sugar. The temperature coefficient of the reaction was at least 6. It is considered that the reaction takes place in two stages, a primary combination and secondary changes, only the latter causing discoloration and loss of solubility. Oxygen had but little effect on these changes, but in air " stale " and " gluey " off-flavours developed which were more objectionable than the " heated " or " caramelised " off-flavours of the powder stored in nitrogen. There was also a slow development of an " oxidised " flavour at moisture contents too low for the protein–lactose reaction.

Lea (1948) has shown that lactose and glucose combine in a 1 : 1 ratio with free amino groups of milk protein but sucrose does not. Discoloration only occurs when this reaction takes place. A review of the browning reaction has been published by Lea (1950). Tarassuk and Jack (1948) have shown that browning is a function of temperature of storage and moisture content, and is accompanied by uptake of O_2 and production of CO_2, increase in reducing groups and titratable acidity, and development of caramelised flavour and very marked decrease in solubility. Whitney and Tracy (1949) have shown that the stale flavour component is concentrated in the fat phase in milk powder and (1950) is extracted with the butter fat to the extent of over 90 per cent.

(iii) *Oxygen tension and gas-packing*

Lea *et al.* (1943) have published a comprehensive and detailed report on the gas-packing and storage of milk powder. On the basis of experiments with butter fat and oxygen they estimated that tallowiness occurs when the available oxygen rises to 0·05 ml per g and that oxygen in the free space in a can of powder would become effective at a concentration of 0·5 to 1·5 per cent. The temperature coefficient of oxidative deterioration was found to be 2·2 per 10° C. Peroxide and Kreis estimations were used to follow the oxidative reactions, and both reached a maximum just before all the oxygen was absorbed and then fell sharply. The development of tallowiness could be progressively delayed by increasingly reducing the oxygen content of the tins. At 47° C (accelerated test) " heated " and " cooked " flavours developed slightly, and later " toffee " and " stale " flavours, but these were far less objectionable than the tallowy flavour normally developing. This could be delayed indefinitely by packing in a gas-tight container with an atmosphere of < 2 per cent oxygen. Prolonged storage showed that spray powders incurred no significant loss of solubility and roller powders only a slight loss, unless of high moisture content. This paper gives copious details of the tests and commercial methods for gas-packing and should be consulted by those seriously interested in the subject.

Hetrick and Tracy (1948c) have described a manometric method for determining the gases desorbed from vacuumised whole-milk powder. Exposure of the powder to air before vacuumising resulted in a fall in the oxygen percentage in the sorbed gases.

(iv) *Temperature of preheating*

The beneficial effect of high preheating temperatures for spray powder has been studied by Findlay *et al.* (1946). Sulphydryl compounds, which prevent the development of oxidised flavours, were produced extensively at 190°–200° F (87·7°–93·3° C), slightly at 180° F (82·2° C) and not at all at 160°–170° F (71·1°–76·6° C). White *et al.* (1947) have found that the keeping quality of spray-dried whole-milk powder could be increased by raising the preheating temperature from 160° to 190° F (71·1°–87·7° C) or by adding ethyl gallate (0·06 to 0·08 per cent) to the extent of 1½ to 2½ times at 116·6° F (47° C), 2 to 3 at 98·6° F (37° C) and 3 to 4 at 59° F (15° C). Manus and Ashworth (1948b) have found that preheating of the milk for 30 min. at 170° F (76·7° C) or 10 min. at 180° F (82·2° C) gave powders of good palatability after 10 months storage at 45° F. A treatment of 30 min. at 160° F (71·1° C) gave a powder which deteriorated rapidly at 45° F (7·2° C). All air-packed powders became rapidly oxidised when held at 100° F (37·8° C).

Christensen *et al.* (1951) and Decker *et al.* (1951) have shown that although milk when preheated becomes rancid during concentration, and yields a very rancid powder, pre-heating for 20 minutes at 140° F (60° C) or higher was sufficient to destroy the lipase in the milk. Such pre-heated milk gave a powder which remained free from off-flavours over storage periods up to eight months. Raising the pre-heating temperature from 140° F to 160° F (71·1° C) effected a slight improvement, and from 160° F to 170° F (76·7° C) a marked improvement.

Pre-heating at 170° F was found to be more effective than double nitrogen packing. This latter will prevent oxidised flavour but will still permit a slightly flat flavour to develop when the powder is stored at 85° F (29·5° C).

They have further found that storage at 85° F tends to accelerate peroxide development in powder made from milk inadequately pre-heated. Lea (1946) has described a method for estimating the sulphydryl compounds which are found in milk heated to above 140° F (60° C). Dowden (1948) has described a very useful " spot test " for oxidation in milk powder based on the " NADI " reagent. Oxidised and tallowy powders give a blue colour, the intensity depending on the degree of oxidation.

Neutralisation of milk for powder

Horwitz (1948) has shown that when the sodium content of skim-milk powder is plotted against the chloride content, all the points for neutralised powders lie to the right of the line Na = 0·62, Cl = 0·1, and those for un-neutralised powder lie to the left. He considers that the Hillig method, which is based on the alkalinity of the ash, is rapid but not accurate. Kunkel and Combs (1943) find that the alkalinity of the ash of un-neutralised skim-milk powder varies from 76 to 127, average 99·5, ml 0·1N HCl per 100 g powder. Differences could be detected due to breed but not any due to season. These workers found that Hillig's (1942) method for the detection of neutraliser was satisfactory if the ash alkalinity of the original milk was known. Otherwise considerable amounts of neutraliser can escape detection.

Acharya and Devadatta (1942) have given analytical figures for P compounds in buffalo milk powder as follows: acid-insoluble 22 (casein 20·1 and lipoid 1·9), baryta-insoluble 67·1, non-hydrolysable 7·8, and baryta-soluble 3·1.

The form of lactose in milk powder

The form in which lactose is present in milk powder will depend upon the method of processing and is of some importance in controlling factors such as physical condition (smoothness, etc.), and moisture equilibrium and absorption and so keeping quality. Thus not only the ratio of α- to β-lactose but also whether the lactose is present as a " glass " or a crystalline form are factors to be considered. This problem is still not clearly understood, but there may be advantages in having the lactose present in the final powder as a crystalline β-anhydride. See Troy and Sharp (1930), Tuckey *et al.* (1934), Gane (1939), Sharp and Doob (1941), Tamsa (1944), Schloemer and Catravas (1947) and Choi *et al.* (1946, 1948, 1949a).

CHAPTER 10

BUTTER AND THE EXAMINATION OF BUTTER FAT

(1)—BUTTER

Butter is the substance, containing practically the whole of the fat of the milk from which it is prepared, produced by the continual shaking or beating of cream, or of the milk itself. In England and most other countries the milk of the cow alone is used, but in Egypt, India and other Eastern countries milks of the buffalo and other animals are used extensively. Originally the production of butter by churning was carried out on a small scale and for the private use of the maker. Except under special circumstances this is no longer the case, and the bulk of world production is manufactured in creameries which collect the milk from farms (or have it delivered to them) and where it is converted into butter under conditions which are clean and hygienic.

The butter is usually produced by churning the cream which has been separated from the milk, although occasionally the milk itself is churned. Years ago the cream was removed from the milk, after it had been standing, by hand-skimming; but this is now done by mechanical separators which are highly efficient and which remove practically the whole of the cream.

The exact mechanisms by which cream becomes churned into butter, and indeed the structure of butter itself, are still matters of dispute. The following references may be consulted: Mulder (1949), King (1949), Mohr (1949), and Wiechers and de Goede (1950). Theories of churning are naturally related to our knowledge of the fat-globule membrane and the factors controlling its stability.

Separated milk is defined by the Sale of Milk Regulations, 1939, as milk which contains not less than 8·7 per cent of solids-not-fat. The average composition of separated milk is—

	Per cent
Fat	0·1 (to 0·3)
Lactose	4·9
Proteins	3·6
Ash	0·8
Water	90·6

When butter is freshly produced it consists of a nodular mass which is then worked, usually on butter tables of various types or in special churn-workers, in order to remove excess of water and make the whole into a visibly homogeneous mass. Salt is added as a flavouring agent and also to act as a preservative.

Storch gives the following as the mean composition of butter—

Table 10.1—Composition of butter (*Storch*)

	From fresh cream per cent	From ripened cream per cent
Fat	83·75	82·97
Water	13·03	13·78
Protein	0·64	0·84
Milk-sugar	0·35	0·39
Ash	0·14	0·16
Salt	2·09	1·86

He argues that the milk-sugar must all belong to the buttermilk, which fills the spaces between the fatty portion; and from the composition of the buttermilk he calculates the proportion of water, proteins, and ash belonging to this.

Table 10.2—Calculated composition of butter (*Storch*)

	From fresh cream per cent		From ripened cream per cent	
Fat	83·75		82·97	
Buttermilk	6·95		8·49	
Water		6·31		7·74
Milk-sugar		0·35		0·39
Protein		0·23		0·29
Ash		0·06		0·07
Mucoid substances	7·21		6·68	
Water		6·72		6·04
Protein		0·41		0·55
Ash		0·08		0·09
Salt	2·09		1·86	

A general description of butter-making is given in Davis's *Dictionary of dairying* and a useful survey of published work is included in Cronshaw's *Dairy information*. The standard textbooks are Hunziker's *The butter industry* and Totman, McKay and Larsen's *Butter*. A critical description of continuous butter-making equipment will be found in Wiechers and de Goede's *Continuous buttermaking*. A good survey of the Alfa method is given by Taylor *et al.* (1945), and Mohr (1947a) has reviewed the possibilities of the beater (Fritz) type of butter-machine and discussed possible alternatives to cold storage of butter.

Structure

Fritz and King (1948) in their comprehensive review of the structure of butter, emphasise the importance of the free fat and the effect of different methods of manufacture. Mohr and Baur (1948) describe methods for estimating this " free oil " in butter. The new methods of making butter are critically discussed by Schulz and Schulz (1948). Schulz (1948) has also devised a specific gravity method for measuring the air content of butter. He finds that churn butter contains 3 to 5, Fritz 5 to 10 per cent and Alfa butter no air.

Mohr (1948) has suggested a theory for the formation of butter in the continuous process. The fat globules which, in 80 per cent cream, are packed very closely together, have an inner absorbed layer of lecithin and an outer protein layer, which is part of the continuous or aqueous phase. The solidification of the fat in the worm cooler causes the globules to burst so that the fat becomes the continuous phase and the lecithin and protein become dispersed with the serum in the fat. Bulk cooling of sterilised cream gives only a partial inversion. Zheltakov (1949) postulates that high-fat cream consists of compressed fat globules surrounded by strongly hydrophilic absorption membranes. Rapid cooling and agitation result in bursting of the membranes and liberation of the fat. The higher glycerides begin to crystallise and the liquid fractions to spread over them, leading to separate drops. In a state of rest the crystallisation spreads and leads to solidification of the butter.

Mohr and Hennings (1947) have found that the dielectric constant of butter fat is constant at 3·1–3·2. Values for good butter vary from 5·0 to 7·5. The structure of the butter affects the value, and the dielectric constant cannot therefore be used for measuring the moisture content.

Flavour and deterioration

Waarden (Eilers *et al.* 1947a) in his monograph on the deterioration of butter flavour in cold storage, suggests that oxidised substances are first formed in the aqueous phase and that these then oxidise lipoids at the fat–water interface. The offensive taints may be due to straight chain, unsaturated aldehydes with from four to eight carbon atoms. Hoecker and Hammer (1942) have shown that the serum of butter always contains more acetoin and diacetyl than the fat (concentrations). Salt reduces the difference in concentrations. Grant *et al.* (1948) have used biochemical tests to follow the deterioration of canned and ordinary butter, and draw attention to the importance of protein-decomposition products in the aqueous phase. Flavour was more closely related to chemical tests than to bacteriological counts.

Mohr and Schroeder (1948) were unable to remove the rancid flavour from butter by boiling, clarifying, steam injection, distilling at reduced pressures, washing with acid, alkali or alcohol, or treating with animal charcoal. Vacuum steam distillation removed rancid and fishy, but not tallowy flavours. (A valuable series of technical papers on butter is abstracted on pp. 165–168 of their paper.)

Jensen (1943) found that vacreation of cream at 200°–205° F (93·3–96·1° C) prevented the development of oxidised flavours, whereas a temperature of 185°–190° F (85°–87·7° C) failed to do so. The use of 0·5 per cent starter in neutralised and pasteurised cream improved flavour and did not affect oxidation. Increase of protein from the normal 0·9 to 1·5 per cent did not affect keeping quality. Butter with the keeping quality of sweet cream butter and the aroma of ripened cream butter can be produced by incorporating a little alkaline salt (sodium carbonate and/or phosphate) in the salt (the mixture constituting Virtanen's " AIV " salt) (Nilsen, 1947).

The superior keeping qualities of sweet cream butter have been confirmed by Mohr (1947b) in Germany. Bird and Fabricius (1944) prepared butter having serum pH values of 6·3, 6·8 and 7·1–7·4. The butter of medium acidity scored highest both fresh and after storage. Ballhöfer *et al.* (1947) found little difference between —6° and —10° C as storage temperatures for butter. Quality when fresh was no guide to keeping quality in storage.

Brunner and Jack (1950) have found that cream held at 0°—10° C for 18 hr. had a 48 to 68 per cent degree of solidification of fat, whereas cream freshly cooled had only 20 to 31 per cent. The higher the proportion of solidified fat, the longer is the churning time. The distribution of solid and liquid fat on the globule surface appears to be, however, a more important factor. Musset *et al.* (1950) found that whereas the liquid fat fractions of milk powder deteriorate rapidly, the solid fat fractions were all relatively stable.

Whitney *et al.* (1950) have separated the substances responsible for the stale flavour of butter by low-pressure steam distillation and ether extraction.

Allen (1950) has studied methods for estimating ascorbic acid in butter. He has found that this rapidly becomes oxidised during the melting of the butter so that only about 50 per cent values are obtained. He has elaborated a simple microtitration method, but with the smaller samples an inevitable loss of at least 10 per cent was experienced. The oxidation of ascorbic acid appears to take place by three concurrent reactions, one being catalysed by the copper originally present in the butter, one by added copper and one by added ferric iron. At —13° C added copper is about 200 times as powerful a catalyst as added iron. In unsalted butter of average heavy metal content at this temperature about half the ascorbic acid is oxidised in about 12 hours. At room temperature 50 per cent would be destroyed in about two hours, and copper is then only 40 times as powerful as iron.

Proportion of solids-not-fat to water

Vieth showed that in butter the proportion of solids-not-fat to water remains (so long as no water is added) the same as that in milk, viz. 10 to 100; he gives the following average analyses—

Table 10.3—Percentage composition of butters (*Vieth*)

Designation	Fat	Water	Curd	Salt	$\frac{\text{Curd}}{\text{Water}} \times 100$
English	86·85	11·54	0·59	1·02	5
French, fresh ..	84·77	13·76	1·38	0·09	10*
,, salt ..	84·34	12·05	1·60	2·01	13*
German, salt ..	85·24	12·24	1·17	1·35	10
Danish, ,, ..	83·41	13·42	1·30	1·87	10
Swedish, ,, ..	82·89	13·75	1·33	2·03	10

* Contained boric acid.

The following analyses by Richmond show the average composition of French fresh butter, giving the amount of preservative, and of Australian butter (percentages)—

Designation	Fat	Water	Curd	Salt	Anhydrous borax	Anhydrous boric acid		Commercial preservative
French, fresh	83·92	14·33	1·36	—	0·21	0·18	=	0·65
Australian, salt	84·50	12·70	1·21	1·57	—	—		—

Table 10.4, referring to butters analysed previous to 1900, gives the number of samples in which the water fell between the percentages named. The analyses were made by Vieth, Schnepel, Boseley, Livett, O'Shaughnessy, and Richmond in the Aylesbury Dairy Company's laboratory—

Table 10.4—Variations of water in butter

Percentages of water	English butters		Foreign butters	
	No. of samples	Percentage	No. of samples	Percentage
7 to 8	2	0·3	—	—
8 ,, 9	5	0·8	5	0·4
9 ,, 10	14	2·2	13	1·0
10 ,, 11	26	4·2	51	3·7
11 ,, 12	65	10·4	78	5·7
12 ,, 13	154	24·6	115	8·4
13 ,, 14	182	29·1	395	29·0
14 ,, 15	97	15·5	373	27·4
15 ,, 16	50	8·0	241	17·7
16 ,, 17	21	3·4	71	5·2
17 ,, 18	4	0·6	21	1·5
18 ,, 19	3	0·5	1	0·1
19 ,, 20	2	0·3	—	—
TOTAL	625	—	1,364	—

The above table contains butters of all kinds—fresh, salt, preserved, unpreserved, fresh from churning, and samples which had been kept for various periods.

Variations in percentages of water

The following table (10.5) is taken from a paper by Faber on "Water in Danish Butter".

Table 10.5—Variations of water in butter (*Faber*)

Percentages of water	No. of samples		Percentage of total	
	Summer	Winter	Summer	Winter
9 to 10	1	1	0·0	0·1
10 ,, 11	16	8	0·8	0·4
11 ,, 12	136	20	6·3	1·0
12 ,, 13	335	138	16·8	7·2
13 ,, 14	534	431	26·7	22·3
14 ,, 15	512	562	25·7	29·1
15 ,, 16	287	447	14·1	23·2
16 ,, 17	124	205	6·2	10·6
17 ,, 18	39	95	2·0	4·9
18 ,, 19	13	20	0·7	1·0
Above 19	4	3	0·2	0·2
Total ..	2,001	1,930	—	—
Average ..	14·03%	14·41%	—	—

Table 10.6—Fat in samples of butter, 1914 to 1936

Percentage of fat	1914–1925	1926–1930	1931–1935	1936
94	3	0	0	0
93	4	1	1	0
92	18	1	0	0
91	39	0	1	0
90	193	26	6	1
89	180	23	12	1
88	500	121	68	11
87	371	144	114	10
86	662	199	212	42
85	392	172	207	31
84	294	72	103	42
83	98	19	20	1
82	29	1	5	0
81	11	3	3	0
80	16	0	0	0
Less than 80	18*	0	0	0
TOTALS	2,828	782	752	139

* 79 to 76 per cent 16, and 1 each of 75 and 73 per cent.

For a considerable number of years the fat was estimated by a volumetric process in the Lancashire County Laboratory. The results obtained are given in Table 10.6.

Table 10.7 shows the effect of keeping on the percentage of water contained in the butter; fresh and salt butters, which were all prepared at the Aylesbury Dairy Company, are kept separate.

Table 10.7—Variations of water in butters on keeping

Percentages of water	Percentages of the total number falling between the limits named				
	Fresh butters		Salt butters		
	Less than 12 hours old	24 to 48 hours old	Less than 12 hours old	12 to 48 hours old	10 to 30 days old
18 to 19	—	—	1·3	—	—
17 ,, 18	—	—	2·5	—	—
16 ,, 17	1·7	—	15·0	3·8	—
15 ,, 16	10·3	10·0	22·5	5·1	3·6
14 ,, 15	31·3	15·0	25·0	12·6	—
13 ,, 14	32·8	35·0	25·0	34·1	10·7
12 ,, 13	20·7	40·0	7·5	38·0	28·6
11 ,, 12	3·4	—	1·3	5·1	42·9
10 ,, 11	—	—	—	1·3	10·7
9 ,, 10	—	—	—	—	3·6
Average percentage of water	13·79	13·54	14·74	13·33	12·00

Taking butters from twenty-four to forty-eight hours old to represent commercial butter, it is seen that salt butter contains rather less water than fresh butter. The contrary is usually stated; but this is not according to Richmond's experience. Fresh butter loses its water chiefly by evaporation, and it is seen that this loss is small; salt butter also loses water by brine running out. It will usually be noticed that salt butter looks wet on being cut, while fresh butter rarely has this appearance.

Analytical results for Australian butter in the rather dry season of 1940–41 have been reported as follows: colour 2–7 L.B.U. For the fat: M.P. 30·5°–35·2° C, iodine value 29·5–42·9, Reichert value 24·2–33·2, saponification value 221–235·3. Cox and McDowall (1948) obtained weighted monthly average values and ranges for New Zealand butter fats as follows: iodine 36·7 (33·8–40 2), Reichert 30·4 (25·5–32·3), saponification 229·5 (225·5–232·7) and softening point 33·1° (32·2–33·7)°C. Schloemer (1947) has reported that the butyric acid numbers of 962 butter fats from 1928 to 1939 varied from 16·1 to 25·1, average 20·3.

Rheology

Dolby (1941b) has studied the measurement of the hardness of butter and concludes that the " cutting wire " (sectilometer) method is more suitable than he loaded cylinder method. Hardness decreases linearly with increasing temperatures, and also falls after working and then slowly recovers. Of the factors in manufacture likely to affect rheological properties, only the rapid cooling of cream after pasteurisation was effective, giving a harder butter than when slow cooling was employed. He has further shown (1949a) that, although the iodine value and softening point of the butter fat are correlated with the softness of the corresponding butter, the iodine value and softening point of the butter fat were independent of each other. He suggests that either increase in iodine value is accompanied by a change in the proportions of the lower saturated acids, so that the average melting point is not changed, or that the softening point is determined more by glyceride structure than by the proportions of the constituent acids. Dolby concludes that by far the greatest cause of variation in butter hardness is differences in the raw material, manufacturing differences accounting for very little.

Buttermilk

The following composition of buttermilk from sweet cream is given by Storch—

	Per cent
Water	89·74
Fat	1·21
Milk-sugar	4·98
Protein	3·28
Ash	0·79

Buttermilk from ripened cream has the following composition—

Table 10.8—Percentage composition of buttermilk (*various observers*)

	Storch	Vieth	Fleischmann
Water	90·93	90·39	91·24
Fat	0·31	0·50	0·56
Milk-sugar	4·58	4·06	4·00
Lactic acid	(?)	0·80	
Protein	3·37	3·60	3·50
Ash	0·81	0·75	0·70

Richmond found the following figures in buttermilks prepared in different ways—

Table 10.9—Composition of buttermilk (*Richmond*)

	Sour cream	Sweet cream	Milk	Separated milk
Specific gravity	1·0314	1·0331	1·0329	1·0355
Water	91·61	90·98	91·13	90·77
Fat	0·50	0·35	0·70	0·10
Milk-sugar	3·40	4·42	3·65	3·93
Lactic acid	0·50	0·01	0·76	0·56
Protein	3·30	3·51	3·28	3·65
Ash	0·65	0·73	0·68	0·79

McDowall and McDowell (1949) have shown that the Udy formula,

$$\text{Percentage fat loss in buttermilk} = \frac{(100 - 1{\cdot}12F)}{F} \times f$$

where F = fat per cent of cream, f = fat per cent in buttermilk,

is more accurate than that of Bird and Derby, as this formula allows for the retention of 10 per cent buttermilk, a figure confirmed by McDowall and McDowell.

Whitman and Tracy (1949) conclude that it is not possible to detect the presence of neutralisers by estimation of the alkalinity of the ash of buttermilk.

Variations of fat

Richmond has found the amount of fat in buttermilk to vary from 0·15 per cent to 5·60 per cent; the last percentage is very unusual, and it is rare to find even as much as 2·0 per cent, percentages higher than this denoting that the churning has been carried out inefficiently.

Ash

The following composition of the ash of buttermilk is given by Fleischmann—

Table 10.10—Composition of ash of buttermilk

	Per cent
Potash, K_2O	24·53
Soda, Na_2O	11·54
Lime, CaO	19·73
Magnesia, MgO	3·56
Phosphoric acid, P_2O_5	29·89
Chlorine, Cl	13·27
Iron oxides, etc.	0·47
	102·99
Less Oxygen = Chlorine	2·99
	100·00

Buttermilk has usually a slightly acid flavour; it does not, however, taste quite like sour skim-milk, but has a distinctive smell and flavour of its own, probably due to diacetyl and related substances.

On microscopic examination it is seen that the fat left is not entirely in globules; there exist many small nuclei consisting of two or more fat globules.

Hodgson (1919) examined 312 samples of buttermilk bought under the Sale of Food and Drugs Acts; of these at least 300 contained added water in amounts varying from 4 to 55 per cent, the water being added during churning. He concluded that it is possible to produce buttermilk in practically every month of the year without the addition of any water whatsoever, but, as it is a matter of custom to add water, he considered that vendors of buttermilk containing over 25 per cent should be cautioned and over 30 per cent prosecuted.

(2) THE EXAMINATION OF BUTTER FAT

The chief characteristic of butter fat is that it contains a high proportion of fatty acids, volatile in steam and soluble in water. This fact has been made use of for the determination of its purity and for its detection in other fats. After Hehner and Angell (1879) had shown that the great difference between butter fat and all other fats was in the comparatively large amount of butyric acid contained in the former, they endeavoured to determine the amount by a distillation method, but they were unable to obtain concordant results and finally gave up the method in favour of the determination of the soluble and insoluble acids. This latter method is not now much used, but experimental details after the American A.O.A.C. are given on p. 498.

Reichert process

Hehner's experiments were continued by Reichert (1879) who was able to overcome the difficulties. Reichert proposed saponifying 2·5 g of butter with caustic soda and alcohol, evaporating off the alcohol, adding 50 ml of water and 20 ml dilute sulphuric acid, and distilling 50 ml in a weak current of air. This method, though Reichert himself calls it Hehner's method, is now known as the Reichert process. He showed that butters took a constant amount of decinormal alkali for neutralisation, while fats and artificial butters took very small quantities (0·3 ml), and coconut oil took about 3 ml; he proposed 14·0 ml as the mean for genuine butters, and 13·0 ml as a limit; he showed also that mixtures of butter and margarine took quantities of 0·1 N alkali equivalent to the amount of butter they contained.

Reichert–Meissl process

Meissl proposed saponifying 5 g of butter fat in a flask of about 200 ml capacity with 2 g of caustic potash and 50 ml of 70 per cent alcohol, and driving off the alcohol on the water-bath. The resulting soap is dissolved in 100 ml of water, and 40 ml of dilute sulphuric acid (1 to 10) are added and the solution distilled with a few small pieces of pumice; 110 ml are collected, filtered, and 100 ml titrated with 0·1 N alkali. In common with Reichert and the earlier experimenters, he used litmus as an indicator, but the superiority of phenolphthalein for this purpose soon became apparent to many. To the number of millilitres of 0·1 N alkali used, one-tenth is added.

Reichert–Wollny process

Wollny (1900), in a now classic memoir, studied the errors of the Reichert–Meissl process; these are—

(1) Error due to the absorption of carbonic acid during the saponification (may amount to + 10 per cent).

(2) Error due to the formation of esters during saponification (may amount to — 8 per cent).

(3) Error due to the formation of esters during the distillation (may amount to — 5 per cent).

(4) Error due to the cohesion of the fatty acids during distillation (may in extreme cases amount to — 30 per cent).

(5) Error due to the shape and size of the distilling vessel and to the time of distillation (may vary the results ± 5 per cent).

To avoid these errors he laid down the following method of working—

Five grams of butter fat are weighed into a round flask of about 300 ml capacity, with a neck 2 cm wide and 7 to 8 cm long; 2 ml of a 50 per cent soda solution and 10 ml of 96 per cent alcohol are added, and the flask heated for half-an-hour on the water-bath under a slanting inverted condenser; between the latter and the flask is a T-piece, which is closed, the limb being turned upwards. At the expiration of half-an-hour the limb of the T-piece is opened and turned downwards, and the alcohol distilled off during a quarter-of-an-hour; 100 ml of boiling water are added by the T-piece, and the flask heated on the water-bath till the soap is dissolved. The solution is allowed to cool to 50° or 60°; 40 ml of dilute sulphuric acid (25 ml to a litre—2 ml of soda solution should neutralise about 35 ml of this) and two pieces of pumice the size of peas are added. The flask is at once furnished with a cork, carrying a tube 0·7 cm in diameter having, 5 cm above the cork, a bulb 5 cm in diameter; above this the tube is bent at an angle of 120°, and 5 cm further on again at an angle of 120°; this tube is joined to a condenser by an india-rubber tube. The flask is heated by a very small flame till the fatty acids are all melted, and the flame is then turned up and 110 ml distilled off in from 28 to 32 minutes. The distillate is mixed well, and 100 ml are filtered off through a dry filter, 1 ml of a 0·5 per cent solution of phenolphthalein solution in 50 per cent alcohol added, and the solution titrated with 0·1 N baryta solution. To the figure thus obtained one-tenth is added, and the amount found by a blank experiment subtracted; the blank should not exceed 0·33 ml.

Leffmann and Beam (1891, 1896) proposed the use of glycerol in place of alcohol for saponification. The process is carried out over a naked flame and is very rapid and convenient. It is essential for carrying out the Polenske modification.

Polenske method

The method known as the Reichert–Meissl–Wollny method depends upon the determination of the soluble steam-volatile fatty acids, that of Polenske on the determination of the insoluble steam-volatile fatty acids. The latter idea seems first to have been considered by Salkowski (1887) but was worked out independently by Müntz and Coudon (1905) and by Polenske (1904). The latter has been the method preferred in England, having been introduced by Bolton (1911). The method of Polenske has now completely taken the place of the earlier modifications of the Reichert process. It has been standardised by the Analytical Methods Committee of the Society of Public Analysts in the manner given on p. 493.

When carrying out the process it is most necessary that the exact conditions be complied with. Richmond and Hall (1920) showed that, although a slight difference in temperature of the distillate is not of serious moment, it is most necessary to adhere strictly to the *time* of distillation, particularly in the case of those fats (e.g. coconut oil) which yield high insoluble acid figures. The quantity and size of the pumice are also of serious moment, and Bolton *et al.* (1912) showed that the position of the hole in the side of the still-head is of importance: it should not be more than about 1 cm from the lower end of the stopper. A micro method with appropriate corrections has been worked out by H. Lührig (1927).

The effect of pressure upon the determination was studied by Kirkham (1920) who states that while the Reichert value is a logarithmic function of the pressure, and errors introduced by ordinary variations in the atmospheric pressure are quite small, the Polenske value is a direct function of the pressure and quite serious errors are likely to be introduced. The following figures were found—

Table 10.11—Reichert and Polenske figures at varying pressures (*Kirkham*)

Pressure (mm)	Reichert		Polenske	
	Found	Calculated	Found	Calculated
100	22·34	22·58	0·19	0·19
180	24·43	24·19	0·48	0·48
250	25·57	25·10	0·75	0·73
380	26·93	26·23	1·14	1·19
450	27·13	26·69	1·61	1·44
627	27·60	27·60	2·06	2·07
760	27·99	28·12	2·68	2·55
900	28·17	28·60	—	—
1,000	28·05	28·87	3·40	3·40

Kirkham suggests that where the barometric pressure differs seriously from the normal, the following correction be applied—

$$V - \frac{v\,(P-K)}{p-K},$$

where P is the pressure at which the Polenske value is V, p is the pressure at which the value is v, and K is a constant, or the pressure at which the Polenske value is 0, in this case 45 mm. Such a correction will only become serious when work is carried out at a barometric pressure differing considerably from 760 mm.

The melting point of the insoluble volatile acids obtained in the Reichert–Polenske process has been studied by various workers. It was first definitely suggested by Blichfeldt (1919)—whose work has been extended by Stokoe (1921)—as a means of distinguishing between coconut and palm-kernel oils, and by Gilmour (1921) as a means for the examination of butter fat with a view to determining adulteration. The following tables show the results obtained by Stokoe by his method—

Table 10.12—Mixtures containing coconut and palm-kernel oils

Coconut per cent	Palm-kernel per cent	Average "seeding-point" °C
85	15	11·8
75	25	13·4
60	40	15·5
50	50	16·9
40	60	17·9
25	75	19·5
10	90	21·8

Four samples of coconut oil of different origin gave seeding-points (i.e. temperature of formation of the first crystals on cooling the melted fats) of 9·9° to 11·4°, average 10·75°; a similar number of samples of palm-kernel oil gave seeding-points of 22·0° to 23·2°, average 22·75°.

As pointed out by Stokoe (1921) the method can be readily carried out as an extension of the Reichert–Polenske method, the contents of the melting-point tube being added to the alcoholic solution of insoluble volatile acids.

The method was examined at some length by Gilmour (1925) who found that in mixtures containing only coconut and palm-kernel oils the quantity of either can be estimated to within about 5 per cent of the amount present, but that when the amount of other oils is above 30 per cent, coconut and palm-kernel oils cannot be estimated separately, but can be estimated together with about the same degree of accuracy. Gilmour determined the melting point of the insoluble volatile acids of butter fat obtained in the Blichfeldt (1919) distillation apparatus. Butter fat gave figures of 15·8° to 25·6°, and it was found that butters yielding the higher amounts of volatile acids usually gave lower melting points for the insoluble portion. It was suggested by Gilmour that with butter having a high distillation figure and a low melting point of insoluble volatile acids, adulteration with a fat which does not give rise to volatile acids would result in a reduction of volatile acids in proportion to the amount of adulteration, whilst the melting point would continue to represent the figure that the pure butter would give, and would probably be too low for the reduced total volatile acid figures. This suggestion does not appear to have been followed.

Arnold (1922) carried out experiments with pure acids with the idea of finding which of them contributed materially to the Polenske value. He found that the butyric and caproic acids yield only soluble acids; caprylic and capric give both Reichert and Polenske figures; whilst lauric and the higher acids give only Polenske figures. Of the insoluble acids, capric and lauric are readily volatile, palmitic and stearic volatile only with difficulty, whilst myristic acid stands midway between the two groups. (The word " volatile " is here used of course as indicating volatility in steam, under the conditions of the Reichert–Polenske process.)

Kirschner process

The Reichert–Polenske method was further extended by Kirschner (1905). This method depends upon the solubility of silver butyrate in dilute silver nitrate solution, whilst the silver salts of the higher fatty acids are practically insoluble. The results obtained by this method, when calculated to percentage of butyric acid in the original fat, are generally about 20 per cent above the actual butyric acid content. The Kirschner method is now usually carried out as a continuation of the Reichert–Polenske; it is described on p. 497. The results obtained with butter and other edible fats and their interpretation are considered on p. 235. Cocks and Nightingale (1928) drew attention to the liability of sulphuric acid being volatilised unless the protecting shield is a perfect fit.

Modified Shrewsbury and Knapp method

Most of the likely adulterants of butter contain little or no volatile acids. The exceptions to the general rule are the oils of the coconut family. The main fatty acids contained in oils of this group are lauric and myristic, and many attempts have been made to base a process on the solubility of these acids in dilute alcohol. The first serious attempt was due to Vandam (1901). The most generally used method in England has been that originally introduced by Shrewsbury and Knapp (1910, 1912). This process has been modified by Revis and Bolton (1911) and Elsdon and Bagshawe (1917). Elsdon (1917) combined the method with that of Reichert–Polenske in a manner originally suggested by Fendler (1910). For the revised method, see p. 499.

A number of methods have been suggested for the examination of butter fat and its adulterants which depend upon the varying solubilities of metallic salts of the fatty acids in different solvents. Thus there is the copper salt method of von Morgenstern (1927), the cadmium salt method of Paal and Amberger (1909), the magnesium salt method of Evers (1910) and the barium salt method of Avé-Lallemant (1907). The last method has been used to a not inconsiderable extent and has been found valuable in certain cases. This method is based upon that of König and Hart (1891); it was favourably commented upon by Revis and Bolton (1910). It depends upon the respective solubilities of the barium salts of the fatty acids. The process is described on p. 500, whilst the interpretation of the results obtained is dealt with on pp. 242–243.

The saponification value and iodine value of butter fat are determined in the usual way. The working details are described on pp. 498 and 502.

Unsaponifiable matter

The term "unsaponifiable matter", as used in the examination of fats, connotes those substances which are not hydrolysed by caustic alkali and which are insoluble in water and soluble in ether. As a general rule the amount of such substances occurring in natural fats is small, the usual quantities being of the order of 1 per cent. In the case of vegetable and terrestrial animal oils (including milk fats), the unsaponifiable matter consists largely of higher monohydric alcohols (sterols) which have characteristic properties. Various other substances, including resins, colouring-matter and hydrocarbons, are present in varying small amounts. In certain cases, such as in some fish oils, the amount of hydrocarbons may become considerable. The nature of the alcohols present gives a means of deciding whether a given fat or oil is from an animal or vegetable source. This provides a valuable method for detecting vegetable oil in butter fat. About one-tenth of the unsaponifiable matter of animal fats consists of cholesterol, whilst one-third to one-half of the unsaponifiable matter of vegetable oils consists of phytosterol. The method as now usually carried out is known as the phytosteryl acetate test. The diagnostic property of the test depends upon the fact that the melting-point of cholesteryl acetate is about 114°, whilst that of phytosteryl acetate is usually about 124°. The evidence points to the fact that the phytosterol usually isolated from a vegetable oil is a mixture. The method was first introduced by Bömer. A reaction common to all sterols is the precipitate given by an alcoholic solution with an alcoholic solution of digitonin. The preparation of digitonin has been described by Panzer (1912). It can be obtained commercially, but the price is high and supplies are not always available. The sterol and digitonin combine to form a simple molecular compound, e.g. digitonin cholesteride $C_{55}H_{94}O_{28}.C_{27}H_{46}O$. The compound is insoluble in water, acetone and ether, and nearly insoluble in 95 per cent alcohol (the solubility is 0·014 g per 100 ml at 18°). The compound is formed by free cholesterol and not by its esters.

Steuart (1923) has paid special attention to the test with regard to the examination of butter and margarine. He used the digitonin method. As a result of his work he was of the opinion that an examination of the sterol prepared from a sample of the fat will show definitely whether the fat is of purely animal origin or whether vegetable fat is present, but that the sterol acetates prepared from some vegetable oils contain fractions of lower melting point, which makes it impossible to detect the presence of animal fats in mixtures containing such vegetable fats by an examination of the sterol acetates. Later work has confirmed the soundness of Steuart's opinion. Hawley (1933b) has devised a convenient method for carrying out the test on comparatively small quantities of fat. The two methods are described in detail on pp. 503 and 504.

Other methods

A considerable number of other methods have been proposed for the examination of butter fat. Among these may be noted the "ethyl ester value" of Hanuš, (1907), the distillation method of Fendler (1910), the magnesium salt method of Grossfeld (1928a), the magnesium–copper salt method of Grossfeld (1928b), the "iodine oxidation value" of Alexander (1939), the butyric acid value of Kuhlmann and Grossfeld (1926a), the "A" and "B" values of Kuhlmann and Grossfeld (1926b), the determination of the insoluble volatile acids due to Hoton (1929, 1930), the "xylene number" of van Raalte (1926, 1930) and the determination of the soluble volatile acids and the soluble silver salts in one operation by Blichfeldt (1919).

Atkinson (1928) has attacked the problem in a somewhat novel manner, and it is unfortunate that this work has not attracted the attention it appears to deserve, as further work along these or similar lines might well repay the trouble involved. Atkinson's work was primarily intended to distinguish the butter fats of cows, buffaloes, sheep and goats. Atkinson found that—

(1) When 1 g of saponified fat is distilled with 210 ml of water, the titration value of the non-volatile acids obtained by subtracting that of the volatile acids, expressed in mg of potassium hydroxide, from the saponification value is, in general, lower in the butter fats of sheep and goats than in those of the cow and buffalo.

(2) If the sum of the two values, oleic acid (from the iodine value) and acids insoluble in 62 per cent aqueous alcohol, is subtracted from the non-volatile acids, a figure is obtained (called " residual acids ") which is, within reasonable limits, constant for the fat of all these animals. The addition of beef fat decreases the residual acids by about two units for every 10 per cent of added fat.

(3) THE INTERPRETATION OF BUTTER-FAT ANALYSES

Reichert value

In examining a sample of butter fat the standard test is always the Reichert–Polenske. When the Reichert value exceeds 28 and the Polenske value is in the correct proportion, the butter fat may be accepted as genuine, provided that there are no general grounds to suspect the correctness of such an opinion. It should not be forgotten that the Reichert value may be increased artificially by the addition of such substances as triacetin or tributyrin. These may of course be extracted from the cold fat with 70 per cent alcohol. Rancid fats have been shown to give abnormally high Reichert values (cf. *Y.B.P.*, 1913, 573, and *infra*). When the Reichert value falls between 26 and 28 the sample will generally prove to be genuine, although there is a far greater possibility of foreign fat having been mixed with the sample than there is of finding it, if present. As the Reichert value falls below 26, the probability of adulteration increases; the lower the figure the greater the possibility of adulteration, although a number of undoubtedly genuine butters have been examined which have had Reichert values as low as 17; such samples are, however, quite exceptional.

The final Report of the Departmental Committee on Butter Regulations, 1904, recommended (*inter alia*), with some dissentients, that the figure 24, arrived at by the Reichert–Wollny method (the results given by the Reichert–Polenske method are substantially the same), should be the limit below which a presumption should be raised that the butter fat is not genuine, but this figure has never received legal sanction. It has, however, become a very usual non-official standard in this country.

In order that the reader may have some information concerning the average value given in the Reichert test for butter fat, and of the likely variations from this, a number of tables are given below which contain the results obtained by various workers. Table 10.13 summarises the evidence given to the Departmental Committee.

Table 10.13—Reichert values of genuine butters

Observer	Type of butter	No. of samples	Range	Average
Brownlee	Irish	127	19·5 to 30·8	28·4
Richmond	Various	700	21·2 ,, 35·0	28·4
Allen	Various	—	25·0 ,, 32·0	28·0
Hehner	Various	—	22·0 ,, (?)	29·0
Dutch Gov. Lab.	Dutch	777	21·5 ,, 33·4	—
Rifle	Norwegian	650	21·1 ,, 34·9	29·7
Thorpe	English	357	22·5 ,, 32·6	—
Lewing	Russian	320	24·5 ,, 30·5	—
Russian Gov. Lab.	Russian	352	20·4 ,, 30·5	25·8
van Rijn*	Dutch	428	17·0 ,, 33·0	25·0
Lewkowitsch	Finnish	—	24·0 ,, 32·0	29·5
Wauters	Belgian	755	19·8 ,, 36·9	29·5
Holm and Kvarup	Danish	7,834	22·4 ,, 33·3	—
Laxa	Czechoslovakian	—	24·0 ,, 32·0	—
Hawley	Indian	52	14·7 ,, (?)	23·1
Arup	Irish (winter)	580	20·4 ,, 31·4	—
French and Raymond	Tanganyika	41	25·2 ,, 28·2	—

* Individual cows varied from 16·8 to 40·0.

According to Richmond, the average of the results of different observers shows that, out of 100 samples,

3 will probably yield Reichert–Wollny figures over 30.
86 ,, ,, ,, ,, between 26 and 30.
7 ,, ,, ,, ,, ,, 25 and 26.
4 ,, ,, ,, ,, below 25.

Low Reichert values are usually caused (this refers to the milk of a herd—individual cows may give "abnormal" butter fat throughout their lactation periods) by exposure of the animals to cold or other adverse conditions, or by the fact that the milk has been produced near the end of the lactation period. There is abundant evidence to show that the proportion of volatile acids is appreciably less during the last month or so of lactation. There is some evidence that season has some effect, but this may well be due to the fact that the proportion of calves born is not constant throughout the year. Tables 10.14 and 10.15 give the Reichert values of butters examined in the Lancashire County Laboratory during the years 1926 to 1936. It should be noted that, in general, the original sources are unknown and that the months given are those in which the samples were submitted for analysis and not those in which they were prepared.

Table 10.14—Reichert values of butter fat (*Lancs. C. C.*)

Reichert value	No. of samples		
	1926 to 1930	1931 to 1935	1936
21·0 to 23·9	5	1	0
24·0 ,, 24·9	3	3	1
25·0 ,, 25·9	6	14	3
26·0 ,, 26·9	21	32	6
27·0 ,, 27·9	71	100	20
28·0 ,, 28·9	104	176	25
29·0 ,, 29·9	146	181	36
30·0 ,, 30·9	161	154	23
31·0 ,, 31·9	72	58	16
32·0 ,, 32·9	25	21	9
33·0 ,, 33·9	8	10	0
34·0 ,, 34·9	1	0	0
TOTALS	623	750	139

Table 10.15—Monthly variation of Reichert values (*Lancs. C. C.*)

Reichert value	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
21·0 to 23·9	—	—	—	—	—	1	—	1	1	—	3	—	6
24·0 ,, 24·9	1	—	—	—	—	—	—	2	—	1	1	2	7
25·0 ,, 25·9	3	1	1	—	—	—	1	4	6	2	5	—	23
26·0 ,, 26·9	3	1	1	—	2	2	3	9	8	20	9	1	59
27·0 ,, 27·9	5	3	1	9	4	10	26	31	28	49	19	6	191
28·0 ,, 28·9	20	17	19	31	14	35	25	23	29	49	27	16	305
29·0 ,, 29·9	38	39	39	24	29	37	33	15	23	35	26	25	363
30·0 ,, 30·9	32	44	22	61	44	25	16	13	8	9	33	31	338
31·0 ,, 31·9	17	14	13	28	19	13	8	4	3	3	9	15	146
32·0 ,, 32·9	11	14	6	5	7	3	1	—	—	1	2	5	55
33·0 ,, 33·9	5	5	—	—	5	—	—	1	—	—	2	—	18
34·0 ,, 34·9	1	—	—	—	—	—	—	—	—	—	—	—	1
TOTALS	136	138	102	158	124	126	113	103	106	169	136	101	1512
Average for month	30·00	30·05	29·87	30·03	30·27	29·44	29·01	28·34	28·31	28·30	28·96	29·85	29·39

Results showing the effect of the lactation period on the Reichert figure are given in Table 10.16. They are taken from p. 507 of the *Minutes of Evidence* of the Departmental Committee.

Table 10.16—Effect of period of lactation on Reichert value

	Length of lactation period in months									
	1	2	3	4	5	6	7	8	9	10
Whole year . .	30·9	29·5	29·1	27·7	26·2	27·5	25·9	24·0	26·2	22·0
Oct.-March	31·5	31·8	31·0	30·4	25·4	—	23·3	24·0	26·2	22·0
April-Sept.	29·6	28·8	28·8	27·1	26·9	27·5	28·1	—	—	—

Table 10.17—Variation of Reichert value with quality

Reichert value		Quality	
Range	Average	Range	Average
33·0 to 26·2	28·6	11·1 to 4·6	8·8
25·9 ,, 24·1	24·9	11·4 ,, 2·9	8·3
23·7 ,, 20·0	22·5	10·9 ,, 4·0	8·4

The market value of butter would appear to bear no relationship to the Reichert value of the fat. Thus on pp. 493 and 494 of the *Minutes of Evidence* will be found a number of Reichert values compared with the number of marks given to the character of the butter at the Danish State butter shows. The marks vary from 2·9 to 11·4 (cf. Table 10.17).

The Polenske value

It was originally claimed by Polenske that the figure obtained by his process bore a more or less strict relationship to the Reichert value. His conclusions were, however, drawn from too small a number of samples, and subsequent investigations have shown that a butter of any given Reichert value may have a Polenske value extending over a considerable range.

As a result of his work on the subject, Richmond found that the Polenske figure varies with the Reichert–Wollny figure, and Table 10.18 shows the relation, which is also expressed by the formula $R\text{-}W \times 0{\cdot}033 - 0{\cdot}6155 = \log_{10}(P - 0{\cdot}48)$. This relation is approximately correct for butters and for mixtures with all fats other than those of the coconut family. The maximum allowable Polenske figure may be calculated by substituting 1·0 for 0·48 in the formula.

When the Polenske figure exceeds the maximum given in the above table for the corresponding Reichert figure, an oil of the coconut family is likely to

Table 10.18

Reichert–Wollny figure	Polenske figure		
	Mean	Calc.	Maximum
32	3·2	3·24	3·7
31	3·0	3·04	3·5
30	2·8	2·85	3·3
29	2·7	2·68	3·2
28	2·6	2·51	3·1
27	2·4	2·37	2·9
26	2·3	2·23	2·8
25	2·1	2·14	2·6
24	2·0	2·02	2·5
23	1·9	1·87	2·4
22	1·8	1·77	2·3
21	1·7	1·70	2·2

be present. Coconut oil itself and palm-kernel oil are those most likely to be present, but Babassu and Cohune nut oils have been used. When coconut oil is present the amount may be estimated by means of the equation—

$$\text{Percentage coconut oil} = \frac{P - P'}{14{\cdot}4} \times 100,$$

where P is the Polenske figure and P' is the mean Polenske figure; this latter figure is found from the table for the Reichert figure corresponding to the sum of the Reichert figure found and half the Polenske figure found, or $\mathrm{R} + \frac{P}{2}$. Thus, supposing that an alleged butter fat gave a Reichert value of 23 and a Polenske value of 3·3, then—

$$\text{Percentage coconut oil} = \frac{3{\cdot}3 - 2{\cdot}1}{14{\cdot}4} \times 100 = 8 \text{ per cent}$$

When palm-kernel oil is known to be present, the figure 8·5 should be substituted for 14·4 in the above equation.

In his examination of 580 samples of Irish winter butter, Arup (1924) found a maximum Polenske value of 3·4 in conjunction with a Reichert value of 22·2. For 290 of these samples the results (Table 10.19) are recorded.

Table 10.20 shows the correlation of Reichert and Polenske figures for imported butter in 1950. Lack of samples from the areas mentioned prevented any comparison being made between samples coming from (*a*) North America and (*b*) Australia and New Zealand.

Table 10.19—Reichert–Polenske values (*Arup*)

No. of samples	Reichert	Polenske		
		Average	Maximum	Minimum
15	22	1·50	1·7	1·2
22	23	1·60	1·8	1·4
43	24	1·65	2·0	1·4
56	25	1·70	2·3	1·4
35	26	1·90	2·4	1·5
26	27	1·95	2·9	1.6
22	28	2·05	3·1	1·6
15	29	2·20	2·9	1·8
26	30	2·10	2·9	1·7
30	31	2·25	3·2	1·6

Table 10.20—Imported butter samples: correlation of Reichert and Polenske figures

	Reichert	36	35	34	33	32	31	30	29	28	27	26	25	24	23	
Polenske	1·3												1			1
	1·4									1			1			2
	1·5											1				1
	1·6										1	2	2	1	1	7
	1·7								3	4	2		1			10
	1·8				1				2	8	4	5				20
	1·9							1	5	6	12	2	1	1		28
	2·0					4	1	3	9	17	9			1		44
	2·1							1	10	15	6					32
	2·2				1	2	1	5	10	15	1					35
	2·3			1	3	3	3	7	16	11	2					46
	2·4			3	3	3		12	9	4	1					35
	2·5			1	4	1	3	6	8	7	1					31
	2·6			1	2		4	6	7	2						22
	2·7				2	8	6	4	6	6						32
	2·8			1		5	8	5	5	5	1					30
	2·9				1	7	8	10	4	6	1					37
	3·0				4	7	7	5	17	2						42
	3·1	1			1	9	4	6	13	5						39
	3·2				3	8	8	11	13	4	1					48
	3·3				3	3	7	8	11	5						37
	3·4				1	2		6	8	3	1					21
	3·5					3	1	2	9	2						17
	3·6					2	1	2	1	2						8
	3·7							2								2
	3·8							1								1
	3·9							1								1
		1	—	7	29	67	62	104	166	130	43	10	6	3	1	629

Out of 629 samples of imported butter examined for moisture content only four were found over the legal limit of 16 per cent.

The equations given above hold good only for the fat of cows' milk.

Knowles and Urquhart (1929) draw attention to the high Polenske value of the fat of goats' butter and to the possibility of such material being returned as butter adulterated with coconut oil (compare Table 4.15, p. 100).

The Kirschner value

This value is of primary importance for the detection and determination of small quantities of butter fat in other fats (see Chapter 11, p. 256). It was shown, however, by Bolton *et al.* (1912) and by Cranfield (1915) that the relationship between the Polenske value and the Kirschner value is more sensitive to the addition of coconut oil than that between the Reichert and Polenske values. Richmond states that the presence of coconut oil may be inferred if the Polenske figure is more than 1·0 ml higher than those given in the table below.

Kirschner figure	*Polenske figure*
26	3·2
24	2·6
22	2·1
20	1·6

Cranfield confirms these figures, his values being—

	Polenske figure	
Kirschner figure	*Average*	*Limits*
24 to 24·4	2·65	2·6 to 2·7
23 „ 24	2·4	2·2 „ 2·6
22 „ 23	2·43	1·8 „ 2·9
21 „ 22	2·05	1·7 „ 2·7
20 „ 21	1·65	1·4 „ 2·2
19 „ 20	1·46	1·4 „ 1·7

Both sets of figures agree in giving the approximate relation in butter, $P = (K - 14) \times 0{\cdot}26$, and in the case of Cranfield's individual results the difference never exceeded 0·7 ml.

Richmond was of the opinion that the presence of coconut or some similar oil might be safely assumed if the Polenske value is higher than $(K - 10) \times 0{\cdot}26$, which formula will indeed give correct results in all normal cases. Several observers have, however, found abnormally high Polenske values when the cows have been fed on beetroot leaves or on turnips. The fat of colostrum may have a low Polenske value (Tchetcherov, 1932).

Density

Butter fat, on account of the presence of glycerides of low molecular weight, has a greater density than the fats used for its adulteration. As it is more convenient and exact to take the density of a liquid than of a solid, the fat is almost invariably melted and the density determined at a temperature above its melting point.

The methods of estimating the density have already been discussed under "Specific gravity of milk" and (except that for butter a considerably higher temperature is employed than that at which the density of milk is taken) the same methods are used.

Two questions arise at the outset: At what temperature shall the density of butter be taken? How shall the results be expressed? The experiments of Skalweit have indicated the most favourable temperature. He took the densities of butter and margarine at various temperatures from 35° C to 100° C, using an incubator to keep a constant temperature. His figures are given in Table 10.21—

Table 10.21—Density of butter and margarine at various temperatures (*Skalweit*)

Temperature	Butter	Margarine	Difference
35° C	0·9121	0·9017	0·0104
50° ,,	0·9017	0·8921	0·0096
60° ,,	0·8948	0·8857	0·0091
70° ,,	0·8879	0·8793	0·0086
80° ,,	0·8810	0·8729	0·0081
90° ,,	0·8741	0·8665	0·0076
100° ,,	0·8672	0·8601	0·0071

These figures show clearly that as the temperature rises, the densities of butter and margarine tend to approach one another; the widest difference occurs at 35° C; Skalweit, therefore, recommends that this temperature be adopted as the temperature at which the density of butter should be determined.

In England a large number of determinations have been made by Bell, Allen, Muter, and others at a temperature of 100° F (37·8° C), and this temperature is very near that found by Skalweit to give the largest difference. In America the temperature of 40° C is used to a considerable extent.

Estcourt proposed using the temperature of boiling water (which he found to raise the butter fat to 97·8° C [208° F]), as being easily attained. Allen and others have recommended this temperature, and find no difficulty in bringing the temperature up to 99° C.

Mode of expressing densities

There is a certain amount of confusion as to the manner in which densities are expressed. To ascertain the true density, the weight of a certain volume of fat should be divided by the weight of the same volume of water at the same temperature and multiplied by the density of water at that temperature. This is very rarely done, so that few published figures are true densities.

Muter gives the term "actual density" to the weight of a certain volume of fat divided by the weight of the same volume of water at the same temperature; densities expressed thus are usually denoted by the symbol *D* 37·8°/37·8°

for density at 37·8°, or *D* 35°/35° for density at 35°, and the true density is often expressed as *D* 37·8°/4° or *D* 35°/4°.

It is usual when densities are taken at the temperature of boiling water to express them in a different way. The weight of a certain volume of fat is divided by the weight of water displaced by a piece of glass which occupies the same volume at the same temperature, when it is cooled down to 60° F (15°5° C). This mode of expression may be denoted by the formula *D* 100°/15·5° in glass. Though apparently cumbersome, this method has certain advantages, as the instrument with which the densities are taken can be standardised at 60° F (15·5° C), and can then be used at any temperature without requiring to be re-standardised. It must be remembered that, though the expansion of glass is very nearly constant, it is not quite so, and over a range of 85° C appreciable differences may occur in the expansion of different instruments. If the glass be not well annealed, internal strains are set up, and these may be so accentuated at high temperatures as to cause distortion and change of volume. It will be readily seen that the method of taking the apparent density in glass at the temperature of boiling water is liable to greater experimental error than determinations at lower temperatures, and (as the experiments of Skalweit have shown) that the effect of experimental error is magnified at 100° C owing to there being a smaller difference between the densities of butter and margarine at this temperature than at lower ones. It is desirable not to adopt this method where accuracy is, as it always should be, a desideratum.

On the whole, it seems desirable to adopt 100° F (37·8° C) as the standard temperature at which determinations should be made, because it is sufficiently near Skalweit's minimum to give a large difference between butter and margarine, and because a large number of experiments on genuine butters have already been made at this temperature.

The determination has now not that importance which it once had, on account of the adoption of more useful methods of analysis. In the following table the ranges obtained by various workers at different temperatures have been collected—

Table 10.22—Density of butter fat

Temperature	Range of density	Authority
15·5°/15·5°C . .	0·936 to 0·942	Fryer and Weston
100° F/100° F . .	0·909 ,, 0·914	Bell; Thorpe; Richmond.
40°/40°C	At least 0·905	U.S. Standard Butter.
100°/15·5° C . .	0·865 to 0·689	Allen; Russian Govt.; Richmond.

In Table 10.23 will be found average figures for a number of fats which have been used as butter adulterants.

The refractive index

Before the widespread use of coconut oil and similar products, the refractive index was a useful sorting test, as butters having a refraction figure of less than

Table 10.23—Densities of butter-fat adulterants

Fat	Density		
	100° F/100° F	100°/15·5° C	15·5°/15·5° C
Lard	0·906	0·860	0·936
Tallow	0·903	0·860	0·947
Cotton-seed oil	—	0·872	0·923
Sesame oil	—	0·867	0·923
Soya-bean oil	—	—	0·925
Arachis oil	—	0·863	0·917
Coconut oil	0·917	0·874	0·926
Palm-kernel oil	—	0·873	0·925

about 43·3 at 40° on the Zeiss butyro-refractometer scale ($n = 1·4545$) could be classed as genuine without further examination. The refraction figure for coconut oil is about 36 at 40° ($n = 1·4495$) and that for animal fats about 50 at 40° ($n = 1·4593$), so that mixtures of these would give refraction figures more or less resembling those for pure butter fat. The average range for butter fat is 42·5 to 44·0 ($n = 1·4542$ to $1·4552$), but much higher and lower figures have been obtained, as set out in the following table—

Table 10.24—Refraction figures of butter fats

Observer	Source	No. of samples	Range of refraction	
			Butyro-refractometer	Index of refraction
Thorpe	English	357	39·7 to 45·9	1·4522 to 1·4565
Stein	Russian	26	42·7 ,, 46·2	1·4543 ,, 1·4567
Rifle	Norwegian	650	41·4 ,, 46·4	1·4534 ,, 1·4569
van Rijn	Dutch	95	43·6 ,, 46·7	1·4549 ,, 1·4571
,,	,,	368	41·6 ,, 48·0	1·4536 ,, 1·4580
Richmond	Various	700	41·1 ,, 46·3	1·4532 ,, 1·4568
Bakst and Lenin	Russian	352	42·0 ,, 46·6	1·4538 ,, 1·4570

The results obtained from 887 samples examined in the Lancashire County Laboratory are given below in Tables 10.25 and 10.26.

Table 10.25—Butter-fat refractions (*Lancs. C. C.*)

Refraction at 40° C	YEAR						TOTAL
	1931	1932	1933	1934	1935	1936	
40·0 to 40·4	1	—	—	1	2	3	7
40·5 ,, 40·9	2	3	7	3	3	3	21
41·0 ,, 41·4	7	24	22	36	11	19	119
41·5 ,, 41·9	20	27	26	22	19	17	131
42·0 ,, 42·4	37	20	24	21	38	31	171
42·5 ,, 42·9	23	18	15	20	16	14	106
43·0 ,, 43·4	22	28	17	23	26	20	136
43·5 ,, 43·9	14	17	19	11	15	7	83
44·0 ,, 44·4	15	8	7	18	13	16	77
44·5 ,, 44·9	10	2	2	9	—	3	26
45·0 ,, 45·4	1	—	—	2	—	3	6
45·5 ,, 45·9	—	—	—	1	—	3	4
Total samples	152	147	139	167	143	139	887
Average refraction ..	42·79	42·45	42·37	42·55	42·54	42·64	42·55

Table 10.26—Monthly variations in refraction (*Lancs. C. C.*)

Refraction at 40° C	MONTH												Tota
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
40·0 to 40·4	1	2	—	—	2	—	—	—	—	—	2	—	7
40·5 ,, 40·9	6	3	2	1	2	—	—	—	—	—	2	5	21
41·0 ,, 41·4	25	15	16	26	7	1	—	2	—	—	11	16	119
41·5 ,, 41·9	19	21	15	28	19	5	3	—	—	2	10	9	131
42·0 ,, 42·4	17	25	18	31	26	21	9	3	2	4	10	5	171
42·5 ,, 42·9	10	11	5	9	12	18	17	4	2	10	6	2	106
43·0 ,, 43·4	10	3	2	5	9	15	22	12	13	19	18	8	136
43·5 ,, 43·9	3	2	—	2	2	3	13	13	13	21	7	4	83
44·0 ,, 44·4	7	1	1	—	—	2	7	10	15	24	8	2	77
44·5 ,, 44·9	1	1	—	—	—	—	1	8	5	6	3	1	26
45·0 ,, 45·4	—	—	—	—	1	—	—	—	2	1	2	—	6
45·5 ,, 45·9	—	1	1	—	—	—	—	—	2	—	—	—	4
Total samples	99	85	60	102	80	65	72	52	54	87	79	52	887
Average refraction	42·07	41·96	41·95	41·91	42·12	42·63	43·08	43·31	43·79	43·60	42·65	42·05	42·5

The saponification value

Köttstorfer proposed the utilisation of the fact that butter fat requires more alkali for its complete saponification than most other fats. The determination is carried out as described on p. 498. According to Köttstorfer, the potash absorption varies from 221·5 to 233·0 in genuine butters, with an average of 227·0. His experience has been confirmed by numerous other observers, although the original limits have been somewhat extended. A number of results are given in Table 10.27—

Table 10.27—Saponification values of butter fat

Observer	Source	No. of samples	Range
Richmond	Various	—	220 to 230
Stein	Russian	26	217 ,, 223
Thorpe*	English	347	216 ,, 238
Bakst and Lenin	Russian	243	217 ,, 229
Siegfeld	German (fed on turnips)	—	233 ,, 243
van Rijn	Dutch	368	209 ,, 230

* One sample 214.

A high saponification value usually accompanied a high Reichert value and vice versa, but this is not always the case.

The iodine value

This figure is of importance on account of the fact that its indications are to a certain extent independent of the Reichert and saponification values. It is largely a measure of the amount of oleic glycerides present. The following table gives a range of results by different observers—

Table 10.28—Iodine values of butter fat

Observer	Source	No. of samples	Range
Richmond	Various	—	31·6 to 42·0
Stein	Russian	26	32·8 ,, 44·8
Bakst and Lenin	Russian	—	24·5 ,, 48·5
van Rijn	Dutch	368	30·6 ,, 50·3
Siegfeld	German	—	22·5 ,, 53·3

Arup (1932a) examined several thousand samples of Irish butter and found the following monthly averages for the years 1931 and 1932—

Table 10.29—Mixed average samples: Irish butter fat

Month	Iodine value (Wijs)		Reichert value		Polenske value	
	1931	1932	1931	1932	1931	1932
January	—	39·6	—	27·0	—	2·0
February ..	—	39·5	—	27·7	—	2·0
March	—	38·3	—	29·9	—	2·0
April	—	38·3	—	30·0	—	1·9
May	38·7	38·2	31·4	31·1	2·8	2·3
June	39·3	38·1	30·8	31·9	2·8	2·3
July	40·0	39·2	30·4	31·8	2·3	2·5
August	41·0	41·4	28·6	30·4	2·2	2·1
September ..	41·3	—	27·6	—	2·1	—
October	43·0	—	26·5	—	1·9	—
November ..	42·3	—	25·6	—	1·8	—
December ..	41·3	—	24·7	—	1·8	—

The thiocyanogen value

A later and valuable method for determining the unsaturated character of an oil is the thiocyanogen value of Kaufmann (1925, 1926, 1928, 1929, 1930). This is carried out in a similar manner to the iodine value, the experimental details being given on p. 505. The thiocyanogen value is by no means equivalent to the iodine value, although the two bear some relationship to each other. Fatty acids such as oleic, having one double bond, give the same results by both methods. (For ease in comparison and calculation, the thiocyanogen value is expressed in the equivalent of iodine.) Linoleic acid, which has two double bonds, only absorbs the thiocyanogen radical at one of them, so that the iodine value is twice the thiocyanogen value. β-elaeostearic acid, which has three double bonds, only absorbs thiocyanogen at one of them, whilst other tri-ethenoid acids are not affected by this reagent.

The method has been applied to butter fat by Arup (1932) and Budhalakoti and Mukherji (1935). In order to keep the fat in solution during the whole of the absorption time, Arup carried out his tests at 25° to 26° C in place of the more usual temperature of 16° to 18° C. At the higher temperature there is a tendency for results to be a little lower. The results given in Table 10.30 were obtained.

It will be noticed that the thiocyanogen value is always less than the iodine value. The amounts noted above correspond to percentages of linoleic acid in the fat varying from 3·3 to 4·6. Budhalakoti and Mukherji (1935) found the percentage of linoleic acid in ten samples of Indian ghee to vary from 3·5 to 5·4.

Table 10.30—Thiocyanogen and other values of butter fat (*Arup*)

Description	Iodine value	Thiocyanogen value	Reichert value	Polenske value
Irish	42·4	38·7	21·1	1·7
,, ...	43·0	39·6	22·7	1·6
,, ...	42·0	39·0	21·5	1·5
,, ...	43·2	40·0	22·2	1·6
,, ...	41·8	38·7	20·7	1·4
,, ...	42·1	38·2	21·8	1·4
,, ...	42·5	38·8	21·1	1·6
,, ...	43·0	39·2	22·2	1·4
,, ...	43·0	39·2	22·1	1·5
,, ...	42·6	38·8	22·2	1·5
,, ...	37·0	34·0	31·5	2·8
,, ...	38·3	34·2	31·3	3·2
,, ...	38·1	34·7	30·7	2·4
,, ...	37·1	33·6	32·2	3·3
,, ...	37·3	33·5	31·1	3·1
,, ...	37·1	32·9	30·9	3·1
,, ...	38·7	35·7	31·2	3·1
,, ...	37·5	34·5	31·6	2·8
,, ...	39·0	34·8	30·0	2·8
,, ...	38·0	33·8	32·1	2·9
,, ...	36·8	33·4	31·5	2·8
New Zealand	32·6	28·8	—	—
Danish ..	31·9	28·0	—	—
,, ...	32·5	28·0	—	—
,, ...	30·6	27·6	—	—
Australian ..	34·5	31·2	—	—
Siberian ..	33·8	30·6	—	—
Argentine ..	39·4	35·7	—	—

The Avé-Lallemant value

The earlier commentators on this process have stated that the expression $b-(200+c)$[1] is always negative in the case of butter fat, whilst the addition of either coconut oil or oleo-margarine will yield a positive figure. The results given in Table 10.31 are due to Avé-Lallemant.

Even if it be allowed that a positive value for the expression $b-(200+c)$ definitely indicates that a butter fat is adulterated, it is obvious that a butter fat giving a very low negative value will permit of fairly extensive adulteration before the impurity of the fat may be assumed.

Bolton and Revis (1910, 1911) speak highly of this test, and Bolton in *Fatty Foods* gives a number of results which confirm these conclusions. A number

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[1] See p. 500.

Table 10.31—Results of Avé-Lallemant

	Reichert value	Mg of barium oxide for 1 g of fat			
		Total	(*a*) Insoluble	(*b*) Soluble	(*c*) Difference *b*—(200 + *c*)
Butter fat (fifty samples):					
Highest	32·3	329·6	254·8	76·7	—23·8
Lowest	24·6	300·9	247·4	50·8	— 0·7
Average	28·7	310·7	250·7	60·3	— 9·6
Coconut oil (three samples):					
Highest	9·6	354·1	299·2	57·6	45·1
Lowest	9·0	351·8	296·5	54·1	38·9
Lard (five samples):					
Highest	—	267·7	259·2	10·4	50·3
Lowest	—	265·0	257·9	7·6	46·9
Sesame oil	—	255·2	251·9	3·3	48·6
Cotton-seed oil	—	263·5	256·9	6·6	50·3

of workers have, however, published somewhat different results. Trimen (1913) working with ghee (see p. 252), agreed that the expression *b*—(200 + *c*) was never positive, but stated that the negative values obtained were sometimes so low that a negative value is still given after the addition of considerable quantities of adulterant. Brownlee (1925) found results with Irish butter fats which were not always negative; out of 127 samples twenty gave positive results, the highest being + 10·4.

Arup (1929a) examined a number of samples of Irish winter butter fat and found that the Avé-Lallemant value ranged from + 14·1 to — 13·3. How far such wide variations are due to the difficulty of manipulation of this process it may be somewhat difficult to say, but whatever the reason it is obvious that this method has not the final value that was at one time claimed for it.

Effect of age on butter-fat constants

When butter is kept and becomes rancid, very pronounced changes take place in the composition of the fat. These may be classed under two heads—hydrolysis and oxidation. If butter fat be kept in the dark and out of contact with the air, it keeps indefinitely without change; but in the presence of light and air it becomes oxidised.

The general course of change may be indicated roughly thus—

(1) The fat is partly hydrolysed into fatty acids and glycerol.
(2) The glycerol is oxidised to fatty acids of low molecular weight.
(3) The unsaturated acids are oxidised, forming peroxides and hydroxy acids.

The general effect of these changes is—

(i) The volatile and soluble acids are increased, the soluble in greater proportion than the volatile.
(ii) The insoluble acids are decreased.
(iii) The iodine absorption is lowered.
(iv) The density and refractive index are increased.
(v) The potash absorption is increased.

If the butter has been kept in its natural state, the butter fat obtained on melting may have properties differing materially from those indicated above, owing to the solubility of some of the products in the water still left in the butter. The soluble and volatile acids in the filtered fat may be lowered from this cause, and the insoluble acids increased.

The course of change is not very rapid, and after several weeks the changes are often not very pronounced.

Bell has recorded the following figures for the changes in the insoluble fatty acids; the butter in this case was kept for the times indicated—

No. of weeks kept	12	7	7	6	8	6
Before keeping, per cent ..	87·60	87·80	85·50	87·40	87·72	87·65
After ,, ,, ,, ..	88·97	90·00	85·72	87·97	88·40	88·00

Vieth made analyses showing the change in the insoluble fatty acids produced when butter fat is kept. In each case about a year had elapsed between the two analyses.

	Per cent	*Per cent*	*Per cent*	*Per cent*
Original insoluble fatty acids	87·43	88·33	87·61	87·72
Insoluble fatty acids, after keeping	85·07	85·97	84·41	83·82

Crispo (1911) found that the fats of samples of butter, wrapped in parchment paper and enclosed in tin boxes, showed a decrease in their proportion of volatile acids. The diminution of the Reichert value varied from 2·3 to 12·8. Melted and filtered butter fat kept in flasks closed with ordinary corks gave somewhat inconsistent results. Eight samples out of twelve showed a slight gain, one remained unchanged, and three showed a slight loss.

Arup (1929b) has shown that the Reichert value of the fat of a sample of butter kept in a bottle fitted with a screw cap and cork for a period of nearly six years, fell from the original of 20·7 to 8·0, but other samples kept in a similar manner, although behaving in the same way, did not do so to nearly so great an extent.

Elsdon *et al.* (1931) examined a number of samples of butter fat which had been allowed to stand in glass crystallising basins, either covered with paper in the dark or uncovered on the bench, for nineteen weeks. The results given in Table 10.32 were obtained.

A mixture of samples of butter fat was allowed to stand for nineteen months, uncovered, in a dark cupboard. The Reichert value increased from 28·0 to 28·9, but the Polenske value remained unchanged at 2·1.

The examination of a considerable number of mixtures of fats containing butter fat, coconut and palm-kernel oils and oleo-margarine showed that over a period of two years the Reichert values increased in every case, the Polenske

Table 10.32—Reichert values of old butter fats

No. of sample	Reichert value			Polenske value			Percentage of free acid as oleic		
	Original	In dark	In light	Original	In dark	In light	Original	In dark	In light
1	30·5	31·0	31·2	2·7	3·6	3·7	0·28	0·79	1·29
2	30·1	30·6	30·9	3·2	3·8	4·2	0·33	1·07	1·12
3	30·4	30·3	31·0	3·3	3·4	4·0	0·28	0·39	1·01
4	29·9	29·7	30·6	2·6	2·8	3·4	0·50	0·50	1·01
5	29·9	29·9	30·2	2·7	3·1	3·4	0·45	0·62	1·10
Lard	0·2	—	1·6	0·3	—	0·6	0·62	—	1·58

sometimes increased, and the Kirschner value increased in every case, but that there was no obvious relationship between the increase in the Kirschner value and the increase in the Reichert value. In some cases the increases were marked, corresponding with a considerable increase in the apparent amount of butter fat present.

A number of samples of canned butter were examined after they had been canned for approximately one year. Beyond a considerable increase in the amount of free fatty acids, there was nothing to distinguish the results from those normally obtained with fresh samples.

Anselmi (1932) found that in rancid butter there is a diminution in the Reichert value; when rancidity is accompanied by mould growth there are irregular variations in all the values. Much smaller variations are obtained with melted and filtered butter fat.

Johnson (1933) found that the fat of Gorgonzola cheese often has a low Reichert value, accompanied by a normal Polenske value and a low Kirschner value. Similar results are recorded by Parkes (1911) and for Neufchâtel cheese by Kirsten (1899).

Bog butter

It has long been a custom in Ireland to bury butter in the peat bogs. This may have been done to preserve it or to impart some characteristic flavour. Some of this butter has been forgotten, and samples are occasionally recovered which may have remained buried for several centuries. Analyses have been reported by Radcliffe and Maddocks (1907) and by Arup (1932b). The characteristics given in Table 10.33 were observed.

The fat has been completely hydrolysed to fatty acids, the soluble fatty acids have almost completely disappeared, and in some cases, judged by the mean molecular weight of the fatty acids and the acetyl values, hydroxy derivatives of the higher acids have been formed by oxidation. It is interesting to observe that Arup was able to isolate characteristic crystals of cholesteryl acetate (melting point 115°) by the digitonin method with one re-crystallisation from both his samples.

Table 10.33—Characteristics of Irish bog butter

Constituent or value	Arup		Radcliffe and Maddocks	Hart
	Leitrim	Tyrone		
Water, per cent	1·54	1·10	—	4·63
Curd ,,	1·31	1·64	1·97	0·32
N × 6·39 ,,	0·37	0·16	—	—
Ash ,,	0·10	0·29	—	—
Salt ,,	0·00	0·00	0·00	0·00
Reichert value	0·6	0·5	1·2	1·4
Polenske value	0·7	0·8	—	—
Iodine value	9·2	9·1	10·9	14·1
Acid value	190·7	196·9	201·9	—
Mean molecular wt. of acids	294·2	284·9	—	—
Acetyl value	20·7	10·1	2·2	—
Melting point, °C	45·6	47·9	48·0	44·0
Unsaponified matter, per cent	0·6	0·6	—	—

General relationship of butter-fat constants

Richmond compiled the following table showing the general character of the relationship of the various constants in the case of pure butter fat—

Table 10.34—Relationship between butter-fat constants (*Richmond*)

Reichert value	Polenske value	Kirschner value	Saponification value	Soluble fatty acids	Insol. fatty acids	Mean molecular weight of I.F.A.	Iodine value	Density $\frac{37\cdot8^\circ}{37\cdot8^\circ}$	Zeiss refracto meter at 40°
22·5	1·7	20·0	219·6	4·3	90·1	266·9	45·1	0·9101	44·8
23·5	1·9	21·0	221·4	4·5	89·7	265·5	43·4	0·9104	44·3
24·5	2·1	21·9	223·2	4·7	89·4	265·0	43·0	0·9108	44·3
25·5	2·2	22·4	223·4	4·8	89·3	264·2	40·7	0·9110	44·1
26·5	2·3	22·9	225·4	4·9	88·9	261·9	39·5	0·9113	43·8
27·5	2·45	23·5	226·8	5·2	88·7	261·7	38·8	0·9114	43·4
28·3	2·65	24·2	228·3	5·4	88·4	260·9	37·2	0·9118	42·9
29·5	2·8	24·8	229·9	5·6	88·3	259·6	35·6	0·9120	42·9
30·5	2·95	25·3	231·4	5·8	87·9	259·1	35·0	0·9123	42·5
31·3	3·1	25·9	232·3	5·9	87·9	258·0	34·0	0·9125	42·5
32·6	3·3	27·1	232·6	6·0	87·7	257·8	32·0	0·9130	42·2

Unfortunately, however, such a table is to a considerable extent idealistic; the following actual results due to Faber for 449 Danish butters, arranged in the order of increasing Reichert value, will indicate the likely variations from Richmond's averages—

Table 10.35—Comparative values for butter fat (*Faber*)

Reichert value	Specific gravity	Saponification value	Refraction at 40° C	Iodine value
18·4 to 19·9	862·8 to 864·1	215·2 to 217·9	42·4 to 43·8	42·5 to 47·2
20·0 ,, 20·9	863·6 ,, 864·6	215·4 ,, 219·8	43·0 ,, 44·1	42·6 ,, 49·4
21·0 ,, 21·4	863·5 ,, 864·7	216·7 ,, 222·2	41·0 ,, 44·1	42·3 ,, 48·3
21·5 ,, 21·9	863·6 ,, 864·6	215·4 ,, 219·5	42·8 ,, 44·3	42·5 ,, 49·1
22·0 ,, 22·4	863·7 ,, 864·9	216·6 ,, 220·6	42·6 ,, 44·4	40·9 ,, 47·6
22·5 ,, 22·9	863·6 ,, 865·2	215·8 ,, 220·9	42·3 ,, 44·5	42·0 ,, 49·7
23·0 ,, 23·4	863·7 ,, 865·1	216·4 ,, 222·5	42·0 ,, 44·8	39·6 ,, 49·2
23·5 ,, 23·9	863·6 ,, 865·1	216·2 ,, 223·9	42·1 ,, 44·6	39·5 ,, 49·1
24·0 ,, 24·4	863·7 ,, 865·3	217·1 ,, 224·9	41·3 ,, 44·6	38·6 ,, 49·8
24·5 ,, 24·9	863·1 ,, 865·7	217·6 ,, 224·7	42·0 ,, 44·0	40·2 ,, 47·3

A number of complete analyses of genuine butter fats, having Reichert values of less than 24, have been compiled from the results of Thorpe, Faber, Lenin, Bakst, van Rijn, etc., and are given in Table 10.36—

Table 10.36—Comparative values for butter fat (various authorities)

Reichert value	No. of samples	Saponification value		Iodine value		Refraction at 40° C		Mean mole-cular weight (aver.)
		Aver.	Range	Aver.	Range	Aver.	Range	
23·9 to 23·6	30	219	216 to 222	42·7	36·0 to 46·6	45·3	43·5 to 47·2	264
23·5 ,, 23·1	43	218	209 ,, 225	43·9	34·4 ,, 50·3	45·5	42·9 ,, 47·5	265
23·0 ,, 22·6	14	218	213 ,, 222	44·9	40·7 ,, 46·7	45·9	44·4 ,, 46·7	264
22·5 ,, 22·1	24	217	213 ,, 222	44·3	32·8 ,, 47·6	45·9	43·2 ,, 46·8	267
22·0 ,, 21·6	13	217	214 ,, 222	46·0	44·2 ,, 48·1	45·4	43·5 ,, 46·8	267
21·5 ,, 21·1	17	216	213 ,, 219	45·3	41·9 ,, 47·9	46·3	45·7 ,, 47·8	—
21·0 ,, 20·6	6	216	211 ,, 220	42·0	32·8 ,, 47·9	45·5	43·2 ,, 46·8	267
20·5 ,, 20·0	6	215	213 ,, 216	47·9	44·5 ,, 49·1	47·6	46·3 ,, 47·7	—
19·9 ,, 19·1	4	215	213 ,, 215	47·1	46·6 ,, 47·5	46·4	45·5 ,, 47·3	267
19·0 ,, 17·0	4	213	212 ,, 214	48·8	48·3 ,, 49·2	47·1	—	—

Adriani *et al.* (1947) have found that a high refractive index indicates a soft butter and conversely. They found six factors to have an appreciable effect on the composition of milk fat—

(1) per cent fat
(2) per cent starchy matter in grass
(3) per cent calcium in grass
} giving low refractive index and hard butter;

$$\left.\begin{array}{l}\text{(4) live-weight over daily fat yield}\\ \text{(5) per cent crude protein in grass}\\ \text{(6) per cent chloride in grass}\end{array}\right\}\ \text{giving high refractive index and soft butter.}$$

van der Burg *et al.* (1945) give the following correlation coefficients for Spring butter—

Refractive index and iodine value	+ 0·976
R.I. and thiocyanogen value	+ 0·971
R.I. and Reichert–Meissl value	— 0·764
I.V and T.V.	+ 0·985
I.V. and R.M.V.	— 0·765
T.V. and R.M.V.	— 0·751
Oleic and linoleic acids	+ 0·23

Kruisheer *et al.* (1947) have postulated that the volatile and solid acids in butter fat constitute a " physiological entity " and have put forward provisional equations for the firmness of the butter fat—

Summer

$$\text{firmness} = 30{\cdot}71 \times \frac{58{\cdot}9-\text{iodine value}}{\text{iodine value}-9{\cdot}6} = 30{\cdot}71 \times \frac{48{\cdot}5-\text{refractometer value}}{\text{refractometer value}-35{\cdot}6}$$

Winter

$$\text{firmness} = 30{\cdot}71 \times \frac{59{\cdot}2-\text{iodine value}}{\text{iodine value}-14{\cdot}2} = 30{\cdot}71 \times \frac{48{\cdot}6-\text{refractometer value}}{\text{refractometer value}-37{\cdot}4}$$

Platon and Olsson (1943) obtained a correlation coefficient of $+0{\cdot}953 \pm 0{\cdot}01$ between refractive index and iodine value for Swedish butter fat. The regression equation was $I = 3{\cdot}81R - 128{\cdot}85$. High yielding cows gave fats with lower iodine values than poor yielders. No relationship was apparent between type of feed and iodine value. The use of dichromate as a preservative did not affect refractive index. According to Frye *et al.* (1950) there is a significant correlation between environmental temperature and the iodine and thiocyanogen values of the milk fat. When fed on linseed meal or cracked soybeans over a long period, the cows appeared to adjust themselves to their feed and maintained a fairly constant value. The maximum effect of feed is reached in about 15 days.

Anantakrishnan *et al.* (1946b) have shown that in the fat of buffalo colostrum, the Reichert, Polenske and Kirschner values increase and the iodine values decrease during lactation. Oleic acid falls from 34 per cent on the first day to 22 per cent on the tenth day, while butyric acid increases from 7 to 12 per cent. Mulder (1947b) has claimed that fat in strippings gives lower iodine and refractometer values than that from fore-milk. He found a similar difference between data for gravity cream and centrifugal cream. In a study of 169 samples of goat-milk fat Zeisset and Grossfeld (1942) obtained mean values as follows: refractometer no. 41·3, total volatile acid no. 40.1, butyric acid no. 14.1, residual acid no. 26.0, saponification no. 235.

General considerations in the testing of butter fat

In general, the first test to apply to a butter fat is the Reichert–Polenske. When the result obtained for the Reichert test is from 28 to 33 and the Polenske value corresponds, the fat may be classified as genuine and probably will be.

There is, however, always the possibility of samples being carefully prepared mixtures, and due regard should always be given to appearance, odour, taste and also to the appearance of the fat on melting.

When the Reichert value is found to be between 24 and 28, the possibility of adulteration is increased, but it would be very difficult to prove, especially in the case of mixtures carefully prepared to pass the test. It should not be forgotten, however, that, on the large scale, it would not be a commercial proposition to introduce a small amount of foreign fat into a butter, whilst mixtures made by small vendors will usually give themselves away by their texture or general appearance. It is not often, therefore, that a butter fat having a Reichert value of above 24 will have been adulterated.

When the Reichert value is below 24, even when the Polenske value is proportionately low, the samples should be regarded with considerable suspicion and further examination made. A high Polenske value in comparison with the Reichert value will indicate oils of the coconut group, but the possibility of the presence of milk fats of other animals should not be overlooked (cf. pp. 97, 100). Added vegetable oils can be demonstrated by means of the phytosteryl acetate test, using the method of Sillevoldt (1929) or of Hawley (1933b) as described on p. 504. Appropriate colour tests can then be applied as indicated on p. 510.

It should not be forgotten that the milk fat of animals fed on cotton-seed oil may give slight reactions for this oil, and that many samples of treated cotton-seed oil give either no reaction or a very faint reaction by the usual tests. The same remarks apply to some extent to sesame oil.

Where the phytosteryl acetate test gives a negative result, any adulteration can only be with such animal fats as lard, beef-fats, tallow or hardened whale oil. Hardened oils are generally indicated by the high iodine values of the solid acids obtained by the lead salt–alcohol process. For pure butter fats and other natural fats this is always less than 5. The detection of adulteration with lard, beef-fat, or tallow is a difficult and, in small quantities, an impossible matter. It must never be forgotten that these fats do not contain any fatty acids foreign to butter fat, and that the natural variations of the amount of each fatty acid present in butter fat allow of a considerable proportion of any one being added before adulteration could be proved or even assumed. Moreover, the glycerides themselves are not so characteristic as to suggest any specific test or definite mode of attack. But tests depending upon the nature of the glycerides should be considered along with those depending upon the nature of the fatty acids. There is an urgent need for reconsideration of the whole question of the adulteration of butter fat with body fats. Such an examination might commence with the suggestions of Atkinson (1928) and should deal, as far as possible, with individual fatty acids; it should also include a careful examination of the glycerides, both as a whole and also those which can be separated by fractional cooling or fractional crystallisation. Until such an inquiry has been set on foot, recourse must be had to the methods already available—methods which may (and in some cases will) leave uncertainty after being carried out.

Determinations should be made of the refractive index, the saponification value, the iodine value, and the mean molecular weights of the fatty acids. The last-named method is regarded as extremely useful by Hawley (1940) when determining the neutralisation value of the fatty acids insoluble in water.

The neutralisation value is the number of milligrams of potassium hydroxide required to neutralise one gram of the specially prepared fatty acids. It is suggested that a sample of bulked butter fat (ghee) which has a neutralisation value of less than 209 is almost certainly adulterated, and that if the neutralisation value is less than 210 the sample must be regarded with suspicion. There appears to be some tendency for high Reichert values to be associated with high neutralisation values, but the latter do not run parallel with the former, as does the saponification value.

The Valenta test (Fryer and Weston, 1918) (see p. 506) should be carried out, but this will not, in itself, definitely decide whether the butter fat is adulterated. The thiocyanogen value may give useful information when used in conjunction with the iodine value to give the percentage of linoleic acid. The difference between the iodine value and the thiocyanogen value multiplied by 1·1 gives the percentage of linoleic acid.

Both the miscibility-curve method of Louise (1907, 1909, 1910, 1911) and the method of Bömer (1914), which depends upon the difference between the melting points of the glycerides more insoluble in ether and the respective fatty acids separated therefrom, would still repay further study (Williams, 1940). In the present state of knowledge, the analyst can only carry out those determinations of which he has had experience, and make his decision after comparing the various results obtained with each other and with the results obtained from known genuine butter fats. The mercury-vapour lamp may sometimes give useful information.

Henville and Paulley (1929) consider that the dye of margarine can be extracted by means of ammonia, whilst the colouring-matter of butter is not. They state that 10 per cent of margarine is easily shown.

The method of Christian and Hilditch—determination of fully-saturated glycerides

Christian and Hilditch (1930) have tackled the problem of the examination of glycerides, but from a different angle. They have extended the oxidation-of-fully-saturated-glycerides method so that determinations can be made of the amount of these, using a comparatively small weight of fat of the order of 50 g. In their proposed method they not only estimate the percentage of fully-saturated glycerides present, but also determine their saponification values and melting-points. As any descripton of the process is of necessity rather lengthy and as, in any case, the interested worker will need to see the original paper, experimental details are not reproduced here. It is suggested that the method will estimate coconut oil when present in butter fat; but it may not attract attention for this purpose, on account of the number of less laborious tests which are available. The claim that it will detect the presence of body fats in butter fat is likely to arouse more interest. It should be borne in mind, however, that the indications given by oils of the coconut family are, as is the case with so many other tests, the converse of those given by body fats, so that a butter adulterated with coconut oil and body fat might be expected to give results approximating to those for pure butter. This fact, however, need not invalidate the test, as the presence of coconut oil would be indicated by the Polenske or Shrewsbury and Knapp tests, and could be allowed for in the interpretation of the results obtained by the method of Christian and Hilditch.

Comparison of the results obtained by them with butter fat and body fat shows that the saponification value and melting points of the "primary neutral" portion of the fully-saturated glycerides (i.e. the main ethereal extract of neutral products of oxidation) suggest that quantities of body fat of the order of 10 per cent or more can be detected. Christian and Hilditch state that, so far as is known at present, the normal variations in the composition of butter fat would affect the fully-saturated glycerides content (possibly by several per cent) but would not affect the saponification value or the melting-point of the "primary neutral" portion, and it is the latter and not the former which are significant. The following results were obtained—

Table 10.37—The method of Christian and Hilditch

Determination	Butter fat	Mutton tallow	Beef tallow	Coconut oil	Palm-kernel oil
Fully-saturated glycerides, per cent	31	28	15	83	65
"Primary neutral"—					
Saponification value	243	211	205	264	257
Melting point ° C (closed)	32 to 37	47 to 56	50 to 53	26 to 28	30 to 32
,, ,, (open)	37 ,, 39	53 ,, 54	52	25 ,, 26	30 ,, 31

In the following table results are given which were obtained with mixtures of butter fat and beef tallow—

Table 10.38—Mixtures of butter fat and beef tallow (*Christian and Hilditch*)

Determination	Beef tallow, per cent			
	0	5	10	20
Fully-saturated glycerides, per cent	32·2	30·8	30·6	31·9
"Primary neutral"—				
Saponification value	244·2	243·5	239·7	231·8
Melting-point ° C (closed	34 to 36	33 to 40	33 to 40	35 to 44
,, ,, (open)	37 ,, 39	41	43	44

As the authors of the process remark, the possible variations in the results given by different butter fats require further study, but the indications given in Table 10.38 seem most promising, and appear to demand further consideration. This method should occupy a prominent place in any investigation undertaken with a view to deciding the most valuable method for the detection of body fats in butter fat.

Chemical characteristics of fats from milk of various mammals

Voorst (1947) has described a method for differentiating cow-, sheep- and goat-milk fat, using the butyric, total lower fatty and residual acid numbers. Bagdasargan (1941) has obtained mean values for ewe butter fat as follows: melting point 34·3° C, solidification point 23·0° C, acidity 0·015 per cent, refractivity 44·3, Reichert–Meissl value 25·0, iodine no. 26·4, saponification value 224·3. De la Mare and Shoreland (1944) report that the component fatty acids in sow milk were (per cent): oleic 35, palmitic 28·3, octadecadienoic 14, hexadecenoic 8·8, stearic 6·1, C_{20}– C_{22} 3·6, steam-volatile 2·4 and myristic 1·8. Figures are given for buffalo milk fat by Heiduschka and Cicekdagi (1940) as follows: butyric 4·2, caproic 1·3, caprylic, 0·4, capric 0·0, lauric 3·0, myristic 7·2, palmitic 25·6, stearic 16·2, arachidic 3·2, oleic 35·2, and linoleic 2·0.

Sadgopal (1948) has classified milk fats of various mammals as follows—

(1) Containing butyric acid: (*a*) low Polenske value—cow and buffalo,
 (*b*) high Polenske value—sheep and goat.
(2) Butyric acid absent: ass, camel and mare.
(3) Steam volatile acids absent: elephant and sow.

Butter oil or butter fat

McDowall *et al.* (1942) describe the large-scale production of dry butter fat. Serum was removed first by gravity and then by centrifugation. Moisture was finally removed by vacuum heating. The final fat contained about 0·03 per cent water, 0·02 p.p.m. copper and 0·02–0·15 p.p.m. iron. Stable emulsions were broken by addition of caustic soda. This paper gives many data of scientific and economic interest. Loftus-Hills has also given a description of commercial-scale production (1942). El-Rafey *et al.* (1944) studied the stabilities, as measured by development of peroxide at 79·5° C, of butter oils prepared in various ways. They found that the boiling-off method heating up to 130° C was best. They ascribed this to the absorption of phospholipids and reducing substances from the denatured protein-lipid compounds in the butter.

A review of continental work has been published by Gasser (1943). High temperatures help to break the emulsion and remove rancid odours. Schaffer and Haller (1943) find that butter fat at atmospheric pressure can dissolve at 40° and 60° C respectively, 10·1 and 9·6 ml air, 14·2 and 12·7 ml oxygen and 109·5 and 91 ml carbon dioxide per 100 ml fat.

Ghee

Ghee is defined by the Bombay Adulteration of Ghee Act as a substance made exclusively from butter fat from which the water has been evaporated by heat, and which contains no admixture of any substance not derived from the milk of the cow, buffalo, goat or sheep. In Egypt the analagous product is known as " samna ", " samnah " or " semnah ", and the buffalo is known as the " gammoose ". The properties of buffalo, goat and sheep milk fats are described under these headings on pp. 96 to 102. Table 10.39 contains characteristics of ghee which have been observed by the authors named.

Speaking generally, cow ghee will have approximately the composition of butter fat, although the milk fat of some Indian cows, possibly on account of the poor nature of some of the pasture, tends to have a low Reichert value, particularly in some provinces. The composition of buffalo ghee is similar to that

Table 10.39—Characteristics of ghee

Authority	Reichert value	Polenske value	Saponification value	Iodine value	Refraction at 40°C	Avé-Lallemant value	Acid value
Bolton and Revis	28·4 to 31·5	1·4 to 2·4	224·9 to 229·1	28·1 to 30·8	41·3 to 41·8	−16·1 to −2·7	3·8 to 7·3
A. K. Menon*	18·2 ,, 25·7	—	206·8 ,, 218·3	—	40·6 ,, 44·6	—	1·5 ,, 2·0
K. H. Vakil	20·5 ,, 25·3	—	218·0 ,, 232·2	—	43·4 ,, 44·9	—	1·5 ,, 3·6
S. H. Trimen	24·4 ,, 36·6	1·4 to 6·4	227·3 ,, 236·3	—	41·5 ,, 42·8	−20·0 to −1·3	—
Browning and Parthasarathy†	18·9 ,, 30·2	—	—	—	—	—	0·5 to 0·8
Ghose	29·0 ,, 42·0	—	226·0 to 240·0	—	40·0 to 42·0	—	—
Hogan and Griffiths-Jones	24·5 ,, 37·0	1·0 to 2·8	218·0 ,, 235·0	23·0 to 39·7	40·4 ,, 43·9	—	—
Trimen (goat)	20·8 & 22·9	4·9 & 6·5	224·5 & 231·6	—	40·6 & 43·6	+0·1 & +17·6	—
Trimen (sheep)	22·9 & 26·5	2·8 & 3·1	216·6 & 223·2	—	44·0	+ 7·7 & + 9·7	—
B. B. Brahmachari‡	19·5 to 42·4	—	213·9 to 236·4	25·6 to 41·1	—	—	—

* 1910, *J. Soc. Chem. Ind.*, **29**, 1428. † 1917, *ibid.*, **36**, 118. ‡ 1927, *B.C.A.*, 973B.

of the fat of buffalo butter, and that of sheep and goat ghee similar respectively to that of sheep and goat milk fat.

The fatty acids and component glycerides of cow ghee and buffalo ghee have been studied by Bhattacharya and Hilditch (1931). The vitamin A content of ghee has been studied by Bacharach (1930).

Baker and Tauber (1932) have reported favourably on the use of the mercury-vapour lamp in the examination of ghee. They examined nearly one thousand samples of both genuine and adulterated ghee and claim to have obtained very useful results.

Persai and Barnicoat (1949) have produced evidence to show that of the factors affecting the keeping quality of ghee, the heat-treatment is more important than moisture content, acidity and type of bacterial culture. Temperatures of 110° C and higher gave the best results provided that the butter was heated as quickly as possible and stirred as little as possible. The phospholipid-antioxidants extracted from the curd promoted good keeping quality. Initial acidities and moisture contents appeared to have no significance, and the life of ghee at 38° C could be predicted with a fair accuracy from results obtained at 100° C with a Swift oxidation tester.

Achaya and Banerjee (1945) have suggested the determination of linoleic acid as a test for adulteration of ghee. Godbole and Sadgopal have written a book on *Butter fat* (*ghee*)*: its composition, nutritive value, digestibility, rancidity, adulteration, detection and estimation* (Benares, 1939).

Dolphin fat

The use of dolphin fat as an adulterant of butter and butter-fat has increased considerably recently, especially in some countries such as Italy. It is difficult to detect the presence of dolphin fat in butter-fat but it is not impossible if the necessary analytical figures are obtained. Two oils are obtained from the dolphin, one from the body and one from the jaw, and the usual fat data are given in Table 10.40. It will be seen from this that the addition of dolphin body oil would raise the refractive index and the iodine number, while the addition of dolphin jaw oil would lower the refractive index and the iodine number, and raise the saponification value and the Reichert-Kirschner values (Williams, 1950).

Table 10.40

		Dolphin fats	
	Butter fat	*Body*	*Jaw*
Refractive index 40° C ..	40–46	59·5	28
Saponification value ..	226–232	215	276
Iodine value	33–45	140	28·3
Reichert value	29–34	27	138
Polenske value	1·8–3·2	0·9	1·9
Kirschner value	21–27	—	133
Sp. gr. 15·5° C/15·5° C ..	0·939	0·931	0·923

CHAPTER 11

MARGARINE AND THE EXAMINATION OF MARGARINE FAT

The first butter substitute was made in Paris in 1870, on account of the great scarcity of butter during the siege of the city during the Franco-German War, and was produced by a Frenchman, Mégé-Mouries, as a result of experiments carried out for a prize offered by the French Government. Early methods of production included the artificial digestion of beef-fat with the linings of pigs' and sheep's stomachs and the churning of the fat with milk and a suspension of macerated cows' udders. These methods have no longer any but historical interest, the present methods of imparting a butter flavour being based upon the treatment of milk with suitable bacterial cultures.

To-day, margarine is not usually produced from oils of any one class. Special mixtures are prepared so that a melting-point may be produced suitable to the time of the year when the margarine will be consumed. The other main factor which controls the oils used is the price at which the margarine is to be sold. Legally, in England, margarine fat may contain up to 10 per cent of butter fat, but not more. Generally speaking, margarine fat contains only the traces of butter fat which come from the skim-milk used in its preparation. The reason for restricting the amount of butter fat in margarine fat, which at first sight may seem obscure, is that before the passing of the Sale of Food and Drugs Act, 1899, it was found that margarines were being prepared the fat of which contained quantities of butter fat as great as 80 per cent. Such mixtures, although at the time legally " margarine ", have a commercial advantage only if sold as butter, and they were doubtless intended to be so sold. When the fraud was discovered, the defence could always be that a mistake had been made and that the substitution was accidental. When the 1899 Act was going through Parliament it was thought that, in order to reduce the possibility of fraudulent substitution, butter fat should be entirely excluded from margarine, but as the manufacturers urged that a small percentage of butter fat would always be present from the milk used, the amount of 10 per cent was suggested and agreed to in order to allow for all eventualities. At the time of the passing of the Act, no one had any intention of adding the full allowance of 10 per cent. About the year 1920 a considerable number of margarines containing butter fat in small quantities were on sale which were described as " Margarine mixed with butter " (an illegal description), and margarines the fat of which contained about 10 per cent of butter fat were described as " contains the highest percentage of butter legally possible ". Such misleading and often illegal descriptions are not now in use to any extent, and this is an advantage to trader and consumer alike.

The oils and fats now chiefly used in the preparation of margarine fat are beef-fat, lard, coconut oil, palm-kernel oil, cotton-seed oil, arachis oil, and whale oil—some of these being hydrogenated to a suitable extent. The examination of margarine fat may be undertaken to find whether it has a legal composition, or whether it has been prepared in accordance with a given specification; or several different fats may have to be examined in connection with tendering for supplies. The number of possible oils and fats is so great, and the problem is further complicated by the likely presence of some of these hydrogenated to a greater or lesser extent, that it is frequently only possible to state the particular classes of oil which have been used, leaving the actual members of each class more or less unidentified. The difficulties are further increased by the fact that all natural products are subject to variations in composition, variations which in some cases are not inconsiderable; thus, although average samples of two different oils may have quite distinct analytical figures, yet abnormal samples may so approach each other in composition as to make exact identification difficult if not impossible. For the determination of the approximate composition of a mixture of oils and fat, it is necessary, therefore, to assume that each ingredient has an average composition. It may be impossible to detect admixture of any one ingredient when the amount falls below ten per cent, except where a colour-test is available, or where, as in such cases as butter fat, coconut oil or arachis oil, some characteristic and easily-determined fatty acids are present.

The standard textbook is Clayton's *Margarine* and his *Theory of emulsions and their technical treatment* is also of interest. A recent article by Andersen (1950) gives a good survey of the manufacture and properties of margarine. Technical aspects of margarine manufacture have also been discussed by Arup (1948). See further Andersen's article on "Margarine" in Davis's *Dictionary of dairying*.

In 1941 margarine was defined by the Federal Security Administrator, U.S.A., and permission given to add artificial colouring, sodium benzoate or benzoic acid, vitamin A (at least 9,000 U.S. Pharmacopoeia units per lb), vitamin D, diacetyl, lecithin or glycerides of the usual fatty acids, butter and salt. The U.S. National Research Council (1944) has issued a report dealing with the composition, nutritive value, consumption and legal aspects of margarine.

Corrections for Reichert and Polenske values

In the examination of margarine fat the first determination to be carried out is that of Reichert–Polenske–Kirschner. This will indicate the presence of butter fat and oils of the coconut class. The calculation of the respective amounts of these oils present is not, however, a straightforward process. Cribb and Richards (1911) found that the Reichert–Polenske figures obtained were not numerically proportional to the amount of the oils present, and that the variations were due to the solubility of the " insoluble acids " (Polenske acids) in the soluble acids (Reichert acids). These authors suggested that a fixed amount of 1·9 should be added to the Polenske value and subtracted from the Reichert value, whilst a modified correction for low values was suggested by Arnaud and Hawley (1912). At a later date, Elsdon and Smith (1925) found that the correction factor varied both with the amount of butter fat and of coconut oil. The figure given by the Kirschner value is, however, a straight-line function. The percentage of butter fat may be calculated from the Kirschner and Polenske values by means of the following equations—

Table 11.1—Equations for butter fat in margarine

Polenske value	*Equation*
Less than 2·0	$B = \dfrac{K - 0{\cdot}3}{0{\cdot}235}$
2·0 to 4·5	$B = \dfrac{K - \frac{P}{6} - 0{\cdot}2}{0{\cdot}235}$
4·6 to 7·0	$B = \dfrac{K - \frac{P}{6} - 0{\cdot}1}{0{\cdot}235}$
7·1 to 9·0	$B = \dfrac{K - \frac{P}{7} - 0{\cdot}1}{0{\cdot}235}$
9·1 to 10·0	$B = \dfrac{K - \frac{P}{8} - 0{\cdot}1}{0{\cdot}235}$
10·1 to 12·0	$B = \dfrac{K - \frac{P}{10} - 0{\cdot}1}{0{\cdot}235}$
12·1 to 17·0	$B = \dfrac{K - \frac{P}{10}}{0{\cdot}235}$

where K is the Kirschner value, P the Polenske value, and B the percentage of butter fat.

The amount of palm-kernel oil and coconut oil can then be roughly estimated by an examination of the Reichert and Polenske figures. The observed Reichert –Polenske figures are first corrected for the amount of butter fat indicated by the Kirschner value by the use of simple proportion, and then use is made of Table 11.2 to discover the possible presence of either coconut or palm-kernel oils, or both.

An examination of Table 11.2 will show that the Reichert value due to palm-kernel oil alone (mixed, of course, with other non-coconut type oils in varying proportions) is in all cases numerically equal to or less than the Polenske value, whilst with coconut oil alone the Reichert value is the greater until about 40 per cent of coconut oil is present. The total amount of oils of the coconut class is then determined by the method of Shrewsbury and Knapp, as described on p. 499, and then, assuming the absence of other oils of this class except coconut and palm-kernel, the approximate proportions of coconut and palm-kernel oils can be obtained from Table 11.2.

It was stated above that this method would *roughly* indicate the amount of coconut and palm-kernel oils present. If it is desired to get nearer the actual amounts present, the correction of the observed Reichert and Polenske values must be carried out other than by simple proportion. When mixtures of fats containing coconut oil, palm-kernel oil and butter fat are examined, the Reichert and Polenske values are not straight-line functions; if the amounts present are to be calculated by simple proportion, the observed Reichert and Polenske values must first be corrected by the factors which have been determined by experiment. When the approximate percentages of coconut and palm-kernel oil have been obtained, the appropriate corrections for these amounts are taken from Tables 11.3 to 11.9. Then, using the corrected Reichert and Polenske figures, the amounts of each can be calculated by simple proportion.

Table 11.2—Reichert and Polenske values of coconut and palm-kernel oils

Palm-kernel oil per cent	*Process*	*Percentage of coconut oil*										
		0	10	20	30	40	50	60	70	80	90	100
0	Reichert ..	0·6	2·0	3·3	4·4	5·2	5·7	6·3	6·6	6·9	7·2	7·4
	Polenske ..	0·4	1·4	2·3	3·5	5·1	6·8	9·4	11·8	13·8	15·2	16·8
10	Reichert ..	1·2	2·5	3·6	4·7	5·5	6·1	6·5	6·8	7·0	7·3	—
	Polenske ..	1·2	2·1	3·6	4·8	6·5	8·4	10·6	12·4	14·6	16·6	—
20	Reichert ..	1·7	3·3	4·2	5·0	5·7	6·3	6·6	6·9	7·2	—	—
	Polenske ..	1·7	2·6	4·4	5·4	7·6	9·6	11·5	13·3	15·4	—	—
30	Reichert ..	2·2	3·7	4·7	5·2	5·9	6·5	6·7	7·0	—	—	—
	Polenske ..	2·5	3·6	5·1	7·2	8·8	10·3	12·5	14·4	—	—	—
40	Reichert ..	2·6	4·0	5·0	5·5	6·2	6·6	6·8	—	—	—	—
	Polenske ..	3·2	5·1	6·8	8·4	9·5	11·8	14·0	—	—	—	—
50	Reichert ..	3·1	4·4	5·3	5·8	6·4	6·7	—	—	—	—	—
	Polenske ..	4·3	6·0	8·0	9·3	11·2	13·0	—	—	—	—	—
60	Reichert ..	3·5	4·8	5·6	6·1	6·6	—	—	—	—	—	—
	Polenske ..	5·6	7·6	9·1	10·8	12·6	—	—	—	—	—	—
70	Reichert ..	3·9	5·2	5·9	6·3	—	—	—	—	—	—	—
	Polenske ..	6·2	8·5	9·7	12·3	—	—	—	—	—	—	—
80	Reichert ..	4·3	5·5	6·1	—	—	—	—	—	—	—	—
	Polenske ..	7·4	9·5	11·5	—	—	—	—	—	—	—	—
90	Reichert ..	4·7	5·6	—	—	—	—	—	—	—	—	—
	Polenske ..	8·6	10·6	—	—	—	—	—	—	—	—	—
100	Reichert ..	4·8	—	—	—	—	—	—	—	—	—	—
	Polenske ..	9·7	—	—	—	—	—	—	—	—	—	—

The amount of coconut oil may be calculated from the corrected Polenske value as follows—

$$\text{Coconut oil (per cent)} = \frac{(P' - 0{\cdot}2 - 0{\cdot}03\,B) \times 100}{17{\cdot}6},$$

where P' is the corrected Polenske value, 17·6 is the average Polenske value of coconut oil, and B the percentage of butter deduced from the Kirschner value. The value 0·2, which is subtracted, is to allow for the Polenske value of the non-coconut base; this figure should, of course, alter with the amount of the base present, being slightly lower in the case of a higher Polenske and slightly higher, say 0·3, where the Polenske value is lower, showing little coconut oil to be present.

The amount of coconut oil present having thus been calculated, the correction for the Reichert value may be found from Table 11.5, and the corrected figure used to determine the percentage of butter fat as follows—

$$\text{Butter fat (per cent)} = \frac{(R' - 0{\cdot}065\,C - 0{\cdot}2) \times 100}{28{\cdot}4},$$

where R' is the corrected Reichert value, C is the percentage of coconut oil calculated from the previous equation, and 28·4 is the average Reichert figure for butter fat. This figure can then be compared with the amount of butter fat calculated from the Kirschner value.

Table 11.3—Reichert, Polenske and Kirschner values of mixtures containing butter fat and palm-kernel oil

Palm-kernel oil per cent	*Process*	Percentage of butter fat								
		0			2			10		
		Expt.	*Calc.*	*Diff.*	*Expt.*	*Calc.*	*Diff.*	*Expt.*	*Calc.*	*Diff.*
0	Reichert	0·6			See Coconut-oil Table					
	Polenske	0·4								
	Kirschner	0·2								
20	Reichert	1·8	1·4	− 0·4	2·3	2·0	− 0·3	4·6	4·3	− 0·3
	Polenske	1·7	2·3	0·6	1·7	2·3	0·6	1·9	2·5	0·6
	Kirschner	0·5	0·4	− 0·1	0·9	0·8	− 0·1	2·9	2·6	− 0·3
40	Reichert	2·6	2·3	− 0·3	3·2	2·9	− 0·3	5·2	5·2	0·0
	Polenske	3·2	4·1	0·9	3·2	4·1	− 0·9	3·5	4·3	0·8
	Kirschner	0·6	0·5	− 0·1	1·0	0·9	− 0·1	3·0	2·7	− 0·3
50	Reichert	3·1	2·7	− 0·4	3·6	3·3	− 0·3	5·6	5·6	0·0
	Polenske	4·3	5·1	0·8	4·3	5·1	0·8	4·7	5·3	0·6
	Kirschner	0·8	0·6	− 0·2	1·1	1·0	− 0·1	3·2	2·8	− 0·4
60	Reichert	3·5	3·1	− 0·4	4·0	3·7	− 0·3	6·0	6·0	0·0
	Polenske	5·6	6·0	0·4	5·6	6·0	0·4	6·0	6·2	0·2
	Kirschner	0·9	0·7	− 0·2	1·3	1·1	− 0·2	3·3	2·9	− 0·4
70	Reichert	3·9	3·5	− 0·4	4·4	4·1	− 0·3	6·5	6·4	− 0·1
	Polenske	6·2	6·9	0·7	6·2	6·9	0·7	6·6	7·1	0·5
	Kirschner	0·9	0·8	− 0·1	1·3	1·2	− 0·1	3·5	3·0	− 0·5
80	Reichert	4·3	4·0	− 0·3	4·7	4·6	− 0·1	6·9	6·9	0·0
	Polenske	7·4	7·8	0·4	7·5	7·8	0·3	7·8	8·0	0·2
	Kirschner	0·9	0·8	− 0·1	1·3	1·2	− 0·1	3·5	3·0	− 0·5
90	Reichert	4·7	4·4	− 0·3	5·1	5·0	− 0·1	7·3	7·3	0·0
	Polenske	8·6	8·8	0·2	8·6	8·8	0·2	8·9	9·0	0·1
	Kirschner	0·9	0·9	0·0	1·3	1·3	0·0	3·6	3·1	− 0·5
100	Reichert	4·8	—	—	—	—	—	—	—	—
	Polenske	9·7	—	—	—	—	—	—	—	—
	Kirschner	1·0	—	—	—	—	—	—	—	—

Butter:—R = 28·5; P = 2·2; K = 22·0

Table 11.4—Reichert, Polenske and Kirschner values of mixtures containing butter f and coconut oil

Coconut oil per cent	*Process*	*Percentage of butter fat* 0		2		4		6		8		10	
		Expt.	*Calc.*	*Expt.*	*Calc.*	*Expt.*	*Calc.*	*Expt.*	*Calc.*	*Expt.*	*Calc.*	*Expt.*	*Cal*
0	Reichert	0·2	0·2	0·7	0·8	1·2	1·4	1·8	1·9	2·4	2·5	2·9	3
	Polenske	0·7	0·7	0·8	0·7	0·9	0·8	1·0	0·9	1·1	0·9	1·2	0
	Kirschner	0·2	0·2	0·7	0·7	1·2	1·2	1·7	1·7	2·2	2·2	2·8	2·
5	Reichert	0·9	0·6	1·5	1·2	2·0	1·7	2·7	2·3	3·2	2·9	3·8	3·
	Polenske	1·2	1·6	1·2	1·6	1·6	1·7	1·8	1·7	2·0	1·8	2·2	1·
	Kirschner	0·3	0·3	0·7	0·8	1·2	1·3	1·7	1·8	2·2	2·3	2·6	2·
10	Reichert	1·6	1·0	2·0	1·5	2·7	2·1	3·3	2·7	3·8	3·3	4·2	3·
	Polenske	1·6	2·4	1·8	2·4	1·9	2·4	2·1	2·5	2·3	2·5	2·4	2·
	Kirschner	0·4	0·4	0·8	0·9	1·3	1·4	1·9	1·9	2·4	2·5	2·9	2·
15	Reichert	2·3	1·4	2·7	1·9	3·4	2·5	4·0	3·1	4·4	3·7	4·9	4·
	Polenske	2·2	3·2	2·4	3·3	2·6	3·3	2·8	3·3	3·0	3·4	3·2	3·
	Kirschner	0·5	0·5	0·9	0·9	1·4	1·5	1·9	2·0	2·5	2·5	3·1	3·
20	Reichert	2·9	1·7	3·3	2·3	3·9	2·9	4·5	3·5	5·0	4·0	5·5	4·
	Polenske	2·4	4·0	2·5	4·0	2·8	4·1	3·0	4·1	3·2	4·2	3·3	4·
	Kirschner	0·6	0·5	1·0	1·0	1·5	1·5	2·0	2·0	2·7	2·5	3·3	3·
30	Reichert	4·0	2·5	4·4	3·1	5·0	3·6	5·5	4·2	6·2	4·7	6·7	5·
	Polenske	3·6	5·6	3·7	5·7	3·9	5·7	4·1	5·8	4·4	5·8	4·5	5·
	Kirschner	0·8	0·7	1·3	1·2	1·8	1·7	2·3	2·2	2·8	2·7	3·3	3·
40	Reichert	4·5	3·2	5·1	3·8	5·5	4·4	6·0	4·9	6·4	5·5	6·9	6·
	Polenske	4·8	7·2	5·2	7·3	5·4	7·3	5·6	7·4	5·7	7·4	5·9	7·
	Kirschner	1·0	0·8	1·5	1·3	2·0	1·8	2·5	2·3	3·1	2·8	3·5	3·
50	Reichert	5·1	4·0	5·7	4·5	6·2	5·1	6·6	5·7	7·2	6·3	7·4	6·
	Polenske	6·7	8·9	6·8	8·9	7·0	9·0	7·1	9·0	7·3	9·1	7·4	9·
	Kirschner	1·2	1·0	1·5	1·5	2·0	2·0	2·5	2·5	3·1	3·0	3·7	3·
60	Reichert	5·5	4·7	6·0	5·3	6·3	5·9	6·6	6·4	7·0	7·0	7·5	7·
	Polenske	9·4	10·5	9·6	10·6	9·8	10·6	10·0	10·7	10·2	10·7	10·3	10·
	Kirschner	1·3	1·1	1·7	1·6	2·2	2·1	2·7	2·6	3·3	3·1	3·7	3·
70	Reichert	6·2	5·5	6·6	6·1	7·2	6·7	7·6	7·2	8·1	7·8	8·6	8·
	Polenske	12·3	12·2	12·2	12·2	12·3	12·3	12·4	12·3	12·6	12·4	12·8	12·
	Kirschner	1·3	1·2	1·7	1·7	2·2	2·2	2·7	2·7	3·2	3·2	3·8	3·
80	Reichert	6·9	6·2	7·4	6·8	7·9	7·4	8·4	7·9	8·8	8·5	9·3	9·
	Polenske	14·1	13·8	14·4	13·8	14·5	13·9	14·6	13·9	14·7	14·0	14·8	14·
	Kirschner	1·4	1·4	1·8	1·9	2·3	2·4	2·9	2·9	3·4	3·4	3·8	3·
90	Reichert	7·3	6·9	7·7	7·5	8·3	8·0	8·7	8·6	9·2	9·2	9·7	9·
	Polenske	15·6	15·5	15·7	15·5	15·8	15·5	16·0	15·6	16·0	15·6	16·4	15·
	Kirschner	1·5	1·5	2·1	2·0	2·6	2·5	2·8	3·0	3·2	3·5	3·7	4·

Butter: R = 29·0; P = 2·6; K = 24·8.

Coconut oil: R = 7·7; P = 17·1; K = 1·7.

Table 11.5—Corrections for observed Reichert and Polenske values

Polenske values				Reichert values			
Observed value	Correction to be added. Percentage of butter			Coconut oil per cent*	Correction to be subtracted. Percentage of butter		
	0	5	10		0	5	10
1·0	0·4	− 0·1	− 0·2	0	0·0	0·1	0·2
1·5	0·7	0·0	− 0·2	5	0·3	0·3	0·3
2·0	0·9	0·5	− 0·2	10	0·6	0·5	0·3
2·5	1·5	0·6	0·2	15	0·9	0·9	0·6
3·5	2·0	1·5	0·9	20	1·2	1·0	0·9
5·0	2·4	1·8	1·5	30	1·5	1·4	1·4
7·0	2·2	2·0	1·7	40	1·3	1·1	0·8
8·0	1·7	1·6	1·3	50	1·1	1·0	0·5
9·0	1·2	1·2	0·9	60	0·6	0·4	0·0
10·5	0·5	0·7	0·5	70	0·7	0·5	0·3
12·0	0·0	0·0	− 0·3	80	0·7	0·5	0·3
14·0	− 0·2	− 0·6	− 0·6	90	0·4	0·2	0·0
16·0	− 0·1	− 0·3	− 0·7	100	0·0	—	—

*Calculated from corrected Polenske value.

The chief difference that will be observed between the palm-kernel table and that for coconut oil is the fact that the differences between the observed and calculated values (calculated from the analytical figures of the components of the mixtures by means of simple proportion) are very much less in the case of the former than of the latter. This is shown in the following table—

Table 11.6—Corrections for Reichert and Polenske values

(Add to observed values)

Palm-kernel oil per cent	*Reichert value*			*Palm-kernel oil per cent*	*Polenske value*		
	Percentage of butter fat				*Percentage of butter fat*		
	0	2	10		0	2	10
0				0			
20	—0·4	—0·3	—0·3	20	0·6	0·6	0·6
40	—0·3	—0·3	0·0	40	0·9	0·9	0·8
50	—0·4	—0·3	0·0	50	0·8	0·8	0·6
60	—0·4	—0·3	0·0	60	0·4	0·4	0·2
70	—0·4	—0·3	—0·1	70	0·7	0·7	0·5
80	—0·3	—0·1	0·0	80	0·4	0·3	0·2
90	—0·3	0·1	0·0	90	0·2	0·2	0·1

The Reichert corrections are quite constant, being of the order of 0·3 (to be subtracted from the observed reading) when no butter is present, and rising to 0·0 in the case of mixtures containing 10 per cent of butter. For most palm-kernel margarines, therefore, the subtraction of 0·3 from the observed Reichert value will give a figure from which the percentage of its components may be calculated by simple proportion.

In the case of the Polenske value, however, the correction in most cases is greater and is not so uniform, so that the actual figures must be taken from the above table. The actual amount of palm-kernel oil present in the mixture may then be calculated from the corrected Polenske value by means of simple proportion.

Correction for Kirschner value

The differences between the calculated and observed Kirschner values are given in the following table, due account of the algebraic sign being taken in making the correction to be added to the observed value—

Table 11.7—Corrections for Kirschner values
(Add to observed values)

Palm-kernel oil per cent	*Percentage of butter fat*		
	0	2	10
0			
20	—0·1	—0·1	—0·3
40	—0·1	—0·1	—0·3
50	—0·2	—0·1	—0·4
60	—0·2	—0·2	—0·4
70	—0·1	—0·1	—0·5
80	—0·1	—0·1	—0·5
90	0·0	0·0	—0·5

The corrections here are greater than with coconut oil when the amount of butter is as high as 10 per cent. For all ordinary purposes a correction of 0·1 should be used when the amount of butter is small, this correction rising to 0·4 when the proportion is of the order of 10 per cent. The exact corrections may be taken from Table 11.4.

Table 11.8—Corrections to be subtracted from observed Reichert values

Palm-kernel oil per cent	*Percentage of coconut oil*										
	0	10	20	30	40	50	60	70	80	90	100
0	0·0	0·7	1·3	1·8	2·0	1·7	1·6	1·2	0·9	0·5	0·0
10	0·2	0·8	1·2	1·6	1·7	1·7	1·4	1·0	0·5	0·2	—
20	0·4	1·2	1·4	1·5	1·5	1·5	1·1	0·7	0·3	—	—
30	0·3	1·2	1·5	1·3	1·3	1·2	0·8	0·4	—	—	—
40	0·3	1·0	1·4	1·2	1·2	0·9	0·4	—	—	—	—
50	0·4	1·0	1·2	1·1	1·0	0·6	—	—	—	—	—
60	0·4	1·0	1·1	0·9	0·8	—	—	—	—	—	—
70	0·4	1·0	1·0	0·7	—	—	—	—	—	—	—
80	0·3	0·8	0·8	—	—	—	—	—	—	—	—
90	0·3	0·5	—	—	—	—	—	—	—	—	—
100	0·0	—	—	—	—	—	—	—	—	—	—

Table 11.9—Corrections to be added to observed Polenske values

Palm-kernel oil per cent	*Percentage of coconut oil*										
	0	10	20	30	40	50	60	70	80	90	100
0	0·0	0·6	1·4	1·8	1·9	1·8	0·8	0·1	—0·3	0·0	0·0
10	0·1	0·9	1·0	1·5	1·4	1·1	0·8	0·4	—0·1	—0·5	—
20	0·6	1·3	1·1	1·8	1·2	0·9	0·6	0·4	0·0	—	—
30	0·7	1·2	1·4	0·9	1·0	1·1	0·5	0·3	—	—	—
40	0·9	0·7	0·6	0·6	1·2	0·5	0·0	—	—	—	—
50	0·8	0·7	0·3	0·7	0·4	0·3	—	—	—	—	—
60	0·4	0·0	0·2	0·1	—0·1	—	—	—	—	—	—
70	0·7	0·1	0·5	—0·5	—	—	—	—	—	—	—
80	0·4	0·0	—0·4	—	—	—	—	—	—	—	—
90	0·2	—0·2	—	—	—	—	—	—	—	—	—
100	0·0	—	—	—	—	—	—	—	—	—	—

Determination of butter fat in margarine

It is possible to construct, by means of curves taken from the experimental results, a table which will give at a glance the amount of butter fat present in a margarine fat, if the observed Polenske and Kirschner results are known. Such a table has been worked out and is given as Table 11.10. The horizontal lines in the table are examined until the two observed figures are found together. The figure at the top of the vertical column will then indicate the amount of butter fat present. For example, if we have a fat in which the Polenske is 5·6 and the Kirschner is 2·5, we examine the table until we find the two nearest figures, which are 5·4 and 2·4 respectively—the interpolated value for the percentage of butter fat is then 6·3.

A method for the determination of butter fat in margarine which is somewhat shorter in that only one determination is necessary has been described by Elsdon and Smith (1927), who modified the method of Gilmour (1920) which depends upon the solubility of the volatile acids in a strong solution of sodium chloride. The experimental details of the method are given on p. 498. By this method it has been found that the salt-soluble figure for butter fat approximates to the Kirschner figure, but that the salt-insoluble figure is approximately double the Polenske figure. The experimental figures for the values obtained from known mixtures with coconut or palm-kernel oils are much nearer to the calculated values than those for the corresponding Reichert and Polenske values. Full details are given in the reference cited (Elsdon and Smith 1927). In order that the amount of butter fat may be determined when present in small quantities, the salt-soluble figure due to coconut (or other similar) oil is found from the Table 11.11, the salt-insoluble figure being assumed, as a first approximation, to be due entirely to coconut oil.

The graphical method of Bolton, Richmond and Revis for estimating the proportions of butter fat, coconut oil and palm-kernel oil in a mixture has been simplified by Williams (1949). This utilises the Reichert, Polenske and Kirschner values, but cannot be used for coconut and palm-kernel oils if the oils of other *Palmae* are present.

Table 11.10—Determination of butter fat in margarine

	Percentage of butter fat										
	0	1	2	3	4	5	6	7	8	9	10
Polenske . .	0·4	0·5	0·5	0·6	0·6	0·7	0·8	0·8	0·9	0·9	1·0
Kirschner . .	0·2	0·5	0·7	1·0	1·2	1·5	1·7	2·0	2·2	2·5	2·7
Polenske . .	1·0	1·1	1·1	1·2	1·2	1·3	1·4	1·4	1·5	1·5	1·6
Kirschner . .	0·3	0·6	0·8	1·1	1·3	1·6	1·8	2·1	2·3	2·6	2·8
Polenske . .	2·0	2·1	2·1	2·2	2·2	2·3	2·4	2·4	2·5	2·5	2·6
Kirschner . .	0·5	0·8	1·0	1·3	1·5	1·8	2·0	2·3	2·5	2·8	3·0
Polenske . .	3·0	3·1	3·1	3·2	3·2	3·3	3·4	3·4	3·5	3·5	3·6
Kirschner . .	0·7	1·0	1·2	1·5	1·7	2·0	2·2	2·5	2·7	3·0	3·2
Polenske . .	4·0	4·1	4·1	4·2	4·2	4·3	4·4	4·4	4·5	4·5	4·6
Kirschner . .	0·8	1·1	1·3	1·6	1·8	2·1	2·3	2·6	2·8	3·1	3·3
Polenske . .	5·0	5·1	5·1	5·2	5·2	5·3	5·4	5·4	5·5	5·5	5·6
Kirschner . .	0·9	1·2	1·4	1·7	1·9	2·2	2·4	2·7	2·9	3·2	3·4
Polenske . .	6·0	6·1	6·1	6·2	6·2	6·3	6·4	6·4	6·5	6·5	6·6
Kirschner . .	1·0	1·3	1·5	1·8	2·0	2·3	2·5	2·8	3·0	3·3	3·5
Polenske . .	8·0	8·1	8·1	8·2	8·2	8·3	8·4	8·4	8·5	8·5	8·6
Kirschner . .	1·1	1·4	1·6	1·9	2·1	2·4	2·6	2·9	3·1	3·4	3·6
Polenske . .	10·0	10·1	10·1	10·2	10·2	10·3	10·4	10·4	10·5	10·5	10·6
Kirschner . .	1·2	1·5	1·7	2·0	2·2	2·5	2·7	3·0	3·2	3·5	3·7
Polenske . .	12·0	12·1	12·1	12·2	12·2	12·3	12·4	12·4	12·5	12·5	12·6
Kirschner . .	1·3	1·6	1·8	2·1	2·3	2·6	2·8	3·1	3·3	3·6	3·8
Polenske . .	14·0	14·1	14·1	14·2	14·2	14·3	14·4	14·4	14·5	14·5	14·6
Kirschner . .	1·4	1·7	1·9	2·2	2·4	2·7	2·9	3·2	3·4	3·7	3·9
Polenske . .	15·5	15·6	15·6	15·7	15·7	15·8	15·9	15·9	16·0	16·0	16·1
Kirschner . .	1·5	1·8	2·0	2·3	2·5	2·8	3·0	3·3	3·5	3·8	4·0

Table 11.11—Comparison of salt-soluble and salt-insoluble figures

Salt-soluble	0·3 to 0·5	0·6	0·7	0·8	0·9	1·0	1·1
Salt-insoluble	0·2 ,, 1·0	1·0 to 2	2 to 3·5	3·5 to 4·5	4·5 to 5·5	5·5 to 7	7 to 8·5
Salt-soluble	1·2	1·3	1·4	1·5	1·6	1·7	
Salt-insoluble	8·5 to 10	10 to 11·5	11·5 to 14	14 to 17	17 to 19	19 to 21	

The amount of salt-soluble acidity due to coconut and similar oils, thus found, is subtracted from the observed value, and the difference is the salt-soluble figure due to butter fat. One per cent of butter fat is equivalent to about 0·2 on the salt-soluble figure. The approximate amount of butter fat present in a margarine may be seen at a glance from Table 11.12.

It must be remembered that the tables and equations given above refer to butter, coconut oil and palm-kernel oil of average composition, and calculations made therefrom will give accurate results only when the constituents of the mixtures examined themselves give results which differ little from the average. The calculated composition of a mixture will therefore depart from

Table 11.12—Butter fat in margarine

	Percentage of butter fat										
Process	0	1	2	3	4	5	6	7	8	9	10
Salt-soluble	0·3	0·5	0·8	1·0	1·2	1·5	1·7	1·9	2·1	2·4	2·6
Salt-insoluble	0·4	0·5	0·5	0·6	0·6	0·7	0·7	0·8	0·8	0·9	0·9
Salt-soluble	0·4	0·6	0·9	1·1	1·3	1·6	1·8	2·0	2·2	2·5	2·7
Salt-insoluble	0·5	0·6	0·6	0·7	0·7	0·8	0·8	0·9	0·9	1·0	1·0
Salt-soluble	0·5	0·7	1·0	1·2	1·4	1·7	1·9	2·1	2·3	2·6	2·8
Salt-insoluble	0·6	0·7	0·7	0·8	0·8	0·9	0·9	1·0	1·0	1·1	1·1
Salt-soluble	0·6	0·8	1·1	1·3	1·5	1·8	2·0	2·2	2·4	2·7	2·9
Salt-insoluble	1·5	1·6	1·6	1·7	1·7	1·8	1·8	1·9	1·9	2·0	2·0
Salt-soluble	0·7	0·9	1·2	1·4	1·6	1·9	2·1	2·3	2·5	2·8	3·0
Salt-insoluble	3·0	3·1	3·1	3·2	3·2	3·3	3·3	3·4	3·4	3·5	3·5
Salt-soluble	0·8	1·0	1·3	1·5	1·7	2·0	2·2	2·4	2·6	2·9	3·1
Salt-insoluble	4·0	4·1	4·1	4·2	4·2	4·3	4·3	4·4	4·4	4·5	4·5
Salt-soluble	0·9	1·1	1·4	1·6	1·8	2·1	2·3	2·5	2·7	3·0	3·2
Salt-insoluble	5·0	5·1	5·1	5·2	5·2	5·3	5·3	5·4	5·4	5·5	5·5
Salt-soluble	1·0	1·2	1·5	1·7	1·9	2·2	2·4	2·6	2·8	3·1	3·3
Salt-insoluble	6·5	6·6	6·6	6·7	6·7	6·8	6·8	6·9	6·9	7·0	7·0
Salt-soluble	1·1	1·3	1·6	1·8	2·0	2·3	2·5	2·7	2·9	3·2	3·4
Salt-insoluble	7·8	7·9	7·9	8·0	8·0	8·1	8·1	8·2	8·2	8·3	8·3
Salt-soluble	1·2	1·4	1·7	1·9	2·1	2·4	2·6	2·8	3·0	3·3	3·5
Salt-insoluble	9·3	9·4	9·4	9·5	9·5	9·6	9·6	9·7	9·7	9·8	9·8
Salt-soluble	1·3	1·5	1·8	2·0	2·2	2·5	2·7	2·9	3·1	3·4	3·6
Salt-insoluble	11·5	11·6	11·6	11·7	11·7	11·8	11·8	11·9	11·9	12·0	12·0
Salt-soluble	1·4	1·6	1·9	2·1	2·3	2·6	2·8	3·0	3·2	3·5	3·7
Salt-insoluble	12·5	12·6	12·6	12·7	12·7	12·8	12·8	12·9	12·9	13·0	13·0
Salt-soluble	1·5	1·7	2·0	2·2	2·4	2·7	2·9	3·1	3·3	3·6	3·8
Salt-insoluble	16·5	16·6	16·6	16·7	16·7	16·8	16·8	16·9	16·9	17·0	17·0
Salt-soluble	1·6	1·8	2·1	2·3	2·5	2·8	3·0	3·2	3·4	3·7	3·9
Salt-insoluble	18·5	18·6	18·6	18·7	18·7	18·8	18·8	18·9	18·9	19·0	19·0
Salt-soluble	1·7	1·9	2·2	2·4	2·7	2·9	3·1	3·3	3·5	3·8	4·0
Salt-insoluble	21·0	21·1	21·1	21·2	21·2	21·3	21·3	21·4	21·4	21·5	21·5

the truth to an extent dependent upon the departure from the average of the figures given by the constituents.

The amounts of butter fat and coconut-oil-type oil having been thus determined, the composition of the remainder may be attacked. Appropriate colour tests should be applied, and the saponification value may be used as a check on the amounts of coconut and palm-kernel oils found. The iodine value will give information concerning vegetable oils, whilst hydrogenated oils will be indicated, both as to presence and amount, by the determination of the amount of iso-oleic acid. Sutton *et al.* (1940) prefer the method of Twitchell (1921). Their paper deals with the examination of lard, but many of the methods to which they refer are likely to be useful in the examination of margarine. It is not possible to outline a scheme which will cover all possible mixtures, but the analyst of experience will be able to apply appropriate methods and calculations as indicated by the results obtained. The Bömer (1913) value may be of considerable assistance. The refractive index and the melting point of the fat will be of value, and further evidence may be obtained from microscopical examination of the crystals obtained by crystallisation from ether. Comparison of the results obtained should always be made with mixtures having the composition deduced from the analytical results.

CHAPTER 12

CHEESE

AND FERMENTED MILK PRODUCTS

Cheese is the solid or semi-solid substance formed from the curd of milk. The coagulation of the milk for the production of curd is usually carried out by means of the enzyme *rennin*. This enzyme occurs widely in nature in both plants and animals, but the commercial preparation is obtained from the inner lining of the fourth stomach of the calf.

Apart from cream cheese, which is of course soft, cheese may be roughly divided into two main classes, namely, hard and soft. The difference in texture is in part due to different moisture content, and in part to the different methods of treatment and the various micro-organisms which take part in the ripening process.

The milk is run into large vats, where it is raised to a suitable temperature for the coagulation process. The optimum temperature is 41° C, but this is seldom reached. The usual range of temperature is from 28° C to 35° C, increase in temperature producing a harder and more elastic curd. The time taken for coagulation varies inversely with the temperature and is of the order of half-an-hour for a hard cheese and more than an hour for a soft cheese. The character of the curd depends to a considerable extent upon the acidity of the milk, this being controlled by the use of cultures of lactic-acid-producing bacteria, known as " starters ". For the production of hard cheese and semi-hard cheese the coagulated milk is " scalded ", the scalding being carried out after the curd has been cut into cubes, when hard cheese is being produced. The typical colonial cheese is prepared from pasteurised milk, which produces a mild-flavoured cheese with a somewhat rubbery texture.

Davis (1935) has given an account of the role of physical, chemical and microbiological factors in the ripening of cheese, and has shown that it is of far greater importance to ensure the right conditions for the growth of the desired type of micro-organism than to ensure their presence by artificial inoculation.

Some cheese, such as Cheddar, Gloucester, and Cheshire, is made by pressing the prepared curd into a mould of a shape which is usually characteristic for each variety. Considerable pressure is applied for two or three days, after which the cheese is allowed to ripen under suitable conditions. In the preparation of other cheese the pressing process is omitted, and cracks are left between the pieces of curd along which blue mould is allowed to grow. This type, which includes Stilton and Gorgonzola, is not infrequently opened up by forcing stiff wires into the moulded cheese. The ripening process is then carried out by moulds, rather than bacteria, so producing the characteristic flavours and aromas.

A fairly extensive account of the scientific principles involved in cheese-making and ripening is given in Davis's *Dictionary of dairying* and the same author has given a general and historical account of Cheddar cheese (Davis, 1944). General articles will also be found in *Dairy Industries* and the *Journal of the Society of Dairy Technology*.

The standard textbooks are van Slyke and Price's *Cheese*, Sammis's *Cheesemaking* and Wilster's *Practical Cheddar cheese manufacture* (all American). Methods of making English varieties are given in *Min. Agric. Bull.*, Nos. 43 and 57.

Effect of quality of milk

Czibulka (1942) failed to find any correlation between pH of the soil and cheese quality in milks produced from soils of pH above 4·4; below this value soils tended to produce milk less suitable for cheesemaking.

Standardisation of milk

Brubaker (1944) assumes that in cheese of 37 per cent moisture, 90 per cent of the fat in the milk remains in the cheese, and that for each 0·1 per cent extra fat in the milk, 0·09 per cent fat, 0·04 per cent casein and 0·012 per cent other solids are retained in the cheese. His equation is—

Percentage of fat in dry matter

$$= \frac{0{\cdot}9T}{0{\cdot}9T + 2{\cdot}0 + 0{\cdot}04(10T-30) + 0{\cdot}46 + 0{\cdot}012(10T-30)}$$

where $T =$ percentage of fat in milk, ordinary or standardised.

As cream, not fat as such, is added or subtracted for standardising purposes, the corresponding correction can be applied.

Pettersen (1947) has used the ordinary formol titration for standardising milk for making skim-milk cheese of a minimum fat content. To produce a cheese with at least 10·5 per cent fat in the dry matter, the milk should be standardised to a fat percentage of

$$\frac{(aP - bR)\ 10{\cdot}5}{(100 - 10{\cdot}5)c}$$

where $P =$ per cent protein in milk by formula
$R =$,, ,, solids other than fat and protein
$a = 75$
$b = 85$
$c = 10{\cdot}5$

Ripening

(i) *Proteolysis*

Davies *et al.* (1934) found that oxidants such as KNO_3 raised the O R potential of ripening Cheddar cheese (cf. p. 56) and repressed ripening. Reducing agents such as KCN had no effect. They considered that normally the flora holds the cheese at a sufficiently low E_h for optimum proteolysis. Copper at 36 p.p.m. markedly inhibited ripening. Variation in the amounts of pepsin and rennin appeared to have little effect on ripening. A number of other chemical substances were also found to have little effect (Davies *et al.* 1937).

Peterson *et al.* (1948a) have estimated the proteinase content of Cheddar cheese by digesting a standard suspension of casein with the cheese mush and precipitating the unchanged casein by trichloracetic acid. The optimum pH was found to be 5, with a secondary value at 7 to 8. The addition of reducing agents accelerated proteolysis. They conclude (Peterson *et al.* 1948b) that the proteinases are largely of bacterial origin. Addition of cysteine accelerated protein breakdown. In the later stages of ripening there appears to be more cysteine-activated proteinase in raw milk cheese than in pasteurised milk cheese.

Some varieties of cheese pass rather quickly from a condition of ripeness to that of " going off " or putrefaction. Florentin (1940) has evolved a " coefficient of total amino nitrogen "—

$$\frac{\text{ammonia N} + \text{amino N}}{\text{total N}} \text{ per cent}$$

to describe this state. He gives values as follows—

	Immature	*Ripe*	*Over-ripe*
Camembert ..	5	13	56
Gruyère	—	12	54
Port-Salut ..	—	5	15

Rather surprisingly he states that the coefficients for Brie and Pont l'Evêque do not increase with age.

Block (1951) has published figures for the amino acids in cheese and concentrated milks and finds that these are similar to those in casein. He was able to show the presence of glutamic acid, glycine, alanine, and (in lesser amounts) valine, leucine, ascorbic acid and serine in protein-free cows' milk. There appeared to be a number of peptides in protein-free milk containing glutamic acid and glycine.

In addition to all the amino acids in casein, cheese was found to contain α- and γ-aminobutyric acid and tyramine.

Kosikowsky (1951) has shown that leucines, methionine, valine, alanine, arginine, lysine, threonine, glycine, asparagine, glutamic acid, aspartic acid, phenylalanine, proline, tyramine, tyrosine, α-amino-η-butyric acid, serine, cystine and probably histidine and γ-aminobutyric acid can be identified in the water-soluble protein decomposition products of Cheddar cheese, using paper partition chromatography. He has also shown that many amino acids rapidly appear in the free state in both raw and pasteurised-milk cheese. In raw-milk cheese glutamic acid, leucine-methionine, basic amino acids, valine, and phenylalanine attained high concentrations, and in the pasteurised-milk cheese glutamic acid, basic amino acids, phenylalanine and asparagine were also found to a high degree. He reports interesting differences in the amounts of some amino acids for raw and pasteurised-milk cheese.

Kosikowsky and Dahlberg (1948a) have given data for the concentration of tyramine in a number of varieties of cheese and find that it varies from 0·005 up to 0·17 per cent.

(ii) *Fat breakdown*

Hlynka and Hood (1947) have found values for the fat acidity of Canadian Cheddar cheese of about 1·0 ml 0·1 N NaOH per 10 g. High values were obtained if the milk lipase was activated by vigorous agitation. Most of this lipolysis took place in the first two or three days. Cheese with values of 2·8 or higher was never first-grade, and the higher the value the lower the grading. Peterson *et al.* (1948c) have described a method for the estimation of lipase in cheese. The enzyme is most active at pH 5, but a secondary optimum occurs at pH 6·5 to 7. They further suggest (1948d) that the milk lipase disappears in the making process, as does also the lipase in the rennet. After 5 to 20 days bacterial lipases make their appearance.

Peterson *et al.* (1949) have compared the volatile acids in Cheddar cheese from raw and pasteurised milk at different stages of ripening up to 400 days (Table 12.1).

Table 12.1—Volatile acids formed during 400 days' ripening of Cheddar cheese made from raw and pasteurised milks

	Raw	*Pasteurised*
	μ mols per 100 *g*	
Acetic	200–500	200–400
Butyric	400–500	300–400
Caproic	0–150	0–100
Caprylic	0–250	0–150
Capric	0–150	0–150

Hiscox *et al.* (1941) have further used the higher solubilities of the higher acids in fat to elaborate their analytical methods. Butter-fat takes up 98·6 per cent caprylic, 88 per cent caproic and 36 per cent butyric. In hard cheese and Roquefort there is little lipolysis and most of the acids are water-soluble. In Gorgonzola and Stilton more of the acids are found in the fat fraction.

The use of raw homogenised milk for cheesemaking may lead to rancid flavour (Snyder and Hansen 1942). Csiszár *et al.* (1942) report values of acetylmethylcarbinol from 0·1 to 8·0, average 3·25, mg per 100 g and of diacetyl from 0 to 1·0, average 0·15, mg per 100 g. The values were not related to the quality of the cheese.

Patton (1950b) has isolated pentanone–2, heptanone–2, nononone–2 and methyl ketones, as well as acetone from ether-extracted steam distillates from blue-vein cheese. A similarity between the odour of blue cheese and these ketones was noticed by a number of observers. The methyl ketones are probably formed by β-oxidation from the fatty acids.

Rheology

Davis (1937) has discussed the fundamental rheology of cheese and given some experimental findings.

Scott-Blair (1938) has devised an apparatus for assessing the firmness of cheese curd (coagulum) at the cutting point, and Coppen (1939) has given results obtained in practice. Scott-Blair and Coppen (1940 and 1941) have described a practical test for assessing the condition (degree of shrinkage) of cheese curd. A simplified form has been used in factory conditions (Scott-Blair and Scott-Blair, M.F. 1941). Whitehead (1948) has produced evidence to indicate that fat helps to retain moisture in cheese curd. High fat contents require high scalding temperatures, more dry-stirring and more salt to reduce the moisture to a given content. Curd from a low casein milk (e.g. Friesian) retains moisture more tenaciously than that from a high casein milk (e.g. Jersey). These two effects tend to neutralise each other, but the " casein effect " is usually more powerful than the " fat effect ".

Mocquot (1947) has compared different techniques for estimating the bound water in cheese. Heating and pressing in manufacture have little effect, but salt has a marked effect. Hoecker and Hammer (1943) have found that the salt distribution in curd is practically uniform one day after removal from press (2 days after washing). Scott-Blair has summarised the present position on rheology in the 2nd edition of Davis's *Dictionary of dairying*, and Baron (1952) has published *The mechanical properties of cheese and butter*.

Miscellaneous

The tiny white particles found in canned Cheddar cheese have been identified by Dorn and Dahlberg (1942) as tyrosine. The white particles in mature Cheddar cheese have been shown by McDowall and McDowell (1939) to consist largely of calcium lactate.

A review of cases of food poisoning by cheese has been compiled by Vergue (1948).

Cheese is usually prepared from cows' milk, but that of other animals is used in some cases, such as Roquefort, which is made from ewes' milk. Hirst (1942) has described in detail the manufacture of cheese from pasteurised goats' milk. Goat flavour was noticed by only a few of the consumers. Stewart (1941) has described the collection of ewes' milk for cheesemaking.

An artificial cheese, known as " margarine cheese ", was formerly prepared by incorporating foreign fats of animal or vegetable origin into a skim-milk or separated-milk cheese. The substitution can be detected by removing the fat and subjecting it to the methods of examination given under butter fat.

During the last twenty years or so a new type of cheese preparation has come into more extended use, namely " processed cheese ". This product is obtained by sterilising mild cheese and incorporating emulsifying salts such as sodium phosphate. It was agreed at the eleventh World's Dairy Congress, held in 1937, that the amount of such emulsifying salts should not exceed 3 per cent and that it was desirable to fix a standard for fat (*Soc. Publ. Anal.*, 1939). Palmer and Sly (1944), after considering the usual emulsifying salts, recommend a mixture of 90 per cent sodium citrate and 10 per cent of disodium phosphate. Leather (1949) has identified crystals in processed cheese (reported as " bits of glass ") as calcium tartrate. These were presumably formed by the interaction of calcium in the cheese and tartrate in the emulsifying salt used. A process for the determination of added disodium phosphate is given by I. M. Williams (1927). Methods for the examination of the products of ripening of cheese are given on pp. 517 to 524.

The composition of cheese

Most of the analyses made have included only water, fat, ash, and total nitrogenous substances, either by difference or by estimation of the nitrogen and multiplication of this by a factor. In very few cases has the separation of the nitrogenous matters been attempted, and it is doubtful whether, where this has been done, much definite information as to the character of the products has been obtained. The chemical knowledge of cheese must be pronounced to be in a much less satisfactory condition than that of other milk products.

Tables 12.2 to 12.7 give the proximate composition of various cheeses; they will be useful as showing the most striking differences. Thus, soft

Table 12.2—Composition of cream cheese (per cent)

	Water	Fat	Protein	Lactic acid, etc.	Ash
(1) **English cream cheese** made without rennet					
Vieth	30·66	62·99	4·94	0·26	1·15
Smetham	20·56	80·03	2·99	0·57	0·83
Pearmain and Moor	57·6	39·3	1·9	—	3·4
Richmond	26·5	67·0	4·05	0·71	0·37
Cribb	23·99	73·24	8·29	—	0·33
(2) **Gervais cheese**					
Vieth	43·32	49·18	7·75	0·72	0·39
Stutzer	44·84	36·73	15·48	—	2·95
Richmond	41·0	49·5	4·1	1·12	0·5
(3) **Pommel cheese**					
Richmond	44·9	45·5	7·2	1·16	0·5
(4) **Fancy cheese**					
Cribb	36·61	48·64	15·44	—	1·43

Vieth found the insoluble fatty acids of the fat in English cream cheese to be—

When fresh	87·31
After 1 month	87·12
,, 2 months	88·02
,, 3 ,,	87·96
,, 4 ,,	87·58

expressed as a percentage of the total fatty acids.

cheese contains large amounts of water and small percentages of fat and protein; cheese made from whole milk contains an amount of fat at least equal to the protein; while skim-milk cheese contains usually less fat than protein. In cream cheese the fat greatly exceeds the protein.

Table 12.3—Composition of soft cheese (per cent)

	Water	Fat	Protein	Lactic acid, etc.	Ash
(1) **Brie**					
Duclaux	50·04	27·5	18·32	—	4·12
(2) **Camembert**					
Duclaux	45·24	30·31	19·75	—	4·70
Stutzer	50·90	27·30	18·66	—	3·14
Cameron and Aikman	51·30	21·50	19·00	—	4·70
Leffmann and Beam ..	51·90	21·00	18·90	—	4·70
Pearmain and Moor ..	45·65	22·25	23·10	—	4·25
Muter	48·78	21·35	19·71	0·36	9·80
(3) **Neufchâtel** (or *Bondon*)					
Fleischmann	34·5	41·9	13·0	7·0	3·6
Pearmain and Moor	39·5	24·4	9·4	—	0·7
Muter	55·2	20·8	15·38	1·64	6·98
(4) **Stracchino**					
König (average) ..	39·21	33·67	29·32	—	3·8

Randoin and Vatinel (1951) have given Ca, P and Ca: P ratios for cheese varieties as follows: Livarot 0·714, 0·299, 2·40; Edam 0·777, 0·332, 2·34; Pont l'Evêque 0·564, 0·296, 1·90; St. Paulin (Port Salut) 0·650, 0·360, 1·83; Münster 0·335, 0·186, 1·80; Cantal 0·776, 0·462, 1·70; Gruyère 1·011, 0·605, 1·68; Coulommier 0·205, 0·147, 1·44; Carré de l'Est 0·195, 0·178, 1·15; Camembert 0·154, 0·139, 1·09; Brie 0·184, 0·188, 0·97. The harder the cheese, the lower is the moisture and the higher are the Ca and P contents, and the higher tends to be the Ca: P ratio.

Table 12.4—Composition of hard cheese (per cent)

	Water	Fat	Protein	Lactic acid, etc.	Ash
(1) **Stilton** (made from milk and cream)					
Pearmain and Moor	20·30	44·00	23·70	—	2·75
Cameron and Aikman	35·20	33·97	24·12	—	3·56
Muter	28·60	30·70	35·60	1·08	4·02
König (average) . .	32·07	34·55	26·21	3·32	3·85
(2) **Cheddar**					
Pearmain and Moor	27·19	30·76	29·20	—	4·66 (American)
,, ,, ,,	33·90	29·05	27·37	—	4·05 (English)
Cameron and Aikman	27·20	32·05	36·60	—	4·15 (American)
,, ,, ,,	28·09	22·52	45·75	—	3·64 (English)
Muter	29·70	30·70	35·00	0·90	3·70 (American)
,, ...	33·40	26·60	34·17	1·53	4·30 (English)
König (average) . .	33·89	33·00	27·56	1·90	3·65
(3) **Cheshire**					
Smetham	39·33	30·80	23·70	2·43	3·60
Pearmain and Moor	34·70	33·30	26·10	—	4·30
Leffmann and Beam	30·4	25·5	36·1	—	4·80
(4) **Gruyère** (or *Emmenthal*)					
Fleischmann . .	36·1	29·5	28·0	3·3	3·1
Duclaux	36·00	29·29	30·84	—	3·87
Stutzer	33·01	30·28	31·41	—	5·30
Pearmain and Moor	31·45	30·20	30·00	—	4·20
Cameron and Aikman	37·34	26·47	31·33	—	3·42
Muter	33·20	27·26	33·49	1·35	4·70
Leffmann and Beam	32·0	28·0	35·1	—	4·8
König (average) . .	36·49	28·01	30·83	0·72	3·95
(5) **Cacciocavallo**					
Sartori	19·76	36·71	34·12	3·70	5·60
Spica and de Blasi . .	23·67	25·49	29·25	17·35	4·24

Table 12.5—Composition of skim-milk cheese (per cent)

	Water	Fat	Protein	Lactic acid, etc.	Ash
(1) **Dutch**					
Duclaux	37·31	24·41	32·50	—	5·69
Pearmain and Moor . .	32·90	17·78	30·80	—	6·40
König (average) . .	37·35	24·61	32·40	—	5·65
Muter	42·72	16·30	28·27	1·35	11·36
(2) **Gloucester** . .					
Cameron and Aikman	28·62	23·67	43·54	—	4·17
Pearmain and Moor . .	35·25	25·80	30·05	—	4·80
Muter	37·20	22·80	33·64	1·80	4·56
(3) **Grana**					
Duclaux	32·56	21·75	42·27	—	5·07
König (average) . .	31·33	23·90	35·34	4·17	5·26
(4) **Parmesan** . .					
Duclaux	30·90	26·04	38·42	—	5·45
König (average) . .	31·80	19·52	41·19	1·18	6·31
Pearmain and Moor . .	32·5	17·1	43·6	—	6·2
Cameron and Aikman	27·56	15·95	44·08	—	5·72
(5) **York** (a soft cheese)					
Muter	63·64	15·14	18·50	1·80	0·92
Vieth	68·44	12·89	14·50	2·88	1·29
Richmond	70·5	10·8	13·8	0·85	1·1
(6) **Bondon ?** (evidently a separated milk cheese)					
Cribb	70·66	1·17	25·38	—	3·46

Marquardt and Yale (1941) give data for cheese from the New York State fair as follows—

Table 12.6

	Salt per cent		*Moisture per cent*	
	Range	*Average*	*Range*	*Average*
Various	0·7–1·93	—	33–42	38
Export Cheddar . .	0·7–1·86	1·34	35–41·5	37·2
Home Cheddar . .	0·96–1·73	1·40	33–41	37·4

Zahrndt *et al.* (1944) obtained the following average values—

Table 12.7

		In dry matter	
	Moisture	*Ca*	*P*
Cheddar	35·2	1·14	0·78
Swiss	37·5	1·36	0·87
Edam	37·8	1·32	0·92
Blue-veined	39·0	0·51	0·54
Cottage	77·0	0·31	0·72
Cream	48·4	0·10	0·23

The well-known *Roquefort* cheese, made from sheep's milk, must also be mentioned. Its composition is as shown in Tables 12.8 and 12.9—

Table 12.8—Composition of Roquefort cheese (per cent)

Authority	Water	Fat	Protein	Lactic acid, etc.	Ash
König (average)	36·85	30·61	25·25	1·90	5·39
Pearmain and Moor	29·6	30·0	28·3	—	6·7
Leffmann and Beam	26·5	32·3	32·9	—	4·4
Muter	21·56	35·96	24·52	0·72	10·24

Steiben gives the following figures (per cent)—

Table 12.9

	Water	Fat	Ash	Insoluble protein	Soluble protein
Fresh	49·66	27·41	1·74	13·72	6·93
Month in cellar	36·93	31·23	4·78	5·02	20·77
Old	23·54	40·13	6·27	8·53	18·47

In the above analyses the figures under the term "protein" include true proteins and their products of ripening, and also such products as lactic acid where these are not given separately.

Proteins of cheese

Besides the analyses given on pp. 272 to 276, those shown in Table 12.10, in which an attempt has been made to distinguish between the various constituents, may be noted.

Table 12.10—Detailed composition of cheese (per cent)

	Water	Fat	Ash	NaCl	Protein	Am-moniacal nitrogen	Amino nitrogen	Authority
Cacciocavallo	19·76	36·71	2·34	3·26	34·12	0·0616	0·665	Sartori
,, ...	23·67	25·49	4·24	3·39	23·63	0·0973	0·987	Spica and de Blasi
,, made from sheep's milk and separated milk	22·09	35·90	2·64	3·16	32·57	0·0503	0·609	Sartori
Placentine	29·07	24·74	4·22	5·04	23·71	0·0911	1·171	Spica and de Blasi
Majorcan	28·85	22·15	3·87	3·08	16·84	0·0826	1·045	Spica and de Blasi
Skim cheese	41·63	5·87	0·45	8·10	34·29	0·170	0·983	Carcano
Grana (fresh)	48·37	13·24	3·71	—	31·88	*Lactose* 1·50	*Amino compounds* 1·02	Sartori

	Water	Fat	Ash	Casein	Albumin	Peptone	Amino com-pounds	Ammonia	Authority
Stracchino (fresh) ..	55·02	24·51	2·43	14·26	1·28	0·74	1·54	0·05	Musso, Menozzi & Bignamini
,, (ripe) ..	40·32	30·83	3·75	14·93	0·71	0·86	8·15	0·42	
Emmenthal I	37·59	31·47	4·15	20·38	0·63	0·75	4·20	0·11	,,
,, II	41·22	26·53	4·58	21·90	0·35	0·74	3·79	0·11	,,
Gruyère	26·31	35·59	5·77	20·57	0·63	0·89	7·80	0·26	,,
Gorgonzola	33·37	37·47	3·38	5·66	0·87	1·74	17·52	0·79	,,
Grana	34·37	17·27	5·05	23·45	0·85	0·55	17·69	0·32	,,

Table 12.10—*continued*

	Water	Fat	Ash	Protein	Products of Ripening	Indigestible Protein	Authority
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
Bellelay	37·58	30·05	3·48	24·33	1·37	0·22	Lindt and Muller
Emmenthal	35·70	32·86	5·25	22·17	6·27	0·15	Schulze and Benecke
Glarner	47·02	6·60	10·10	31·76	7·55	0·85	,, ,, ,,

Soft Cheese (*Duclaux*)

	Water	Fat	Insoluble protein	Soluble protein	Salt	Ash	Caseone	Ammonia		Butyric acid
								Free	Combined	
Brie	50·04	27·50	12·90	5·42	3·22	0·90	5·72	0·157	0·233	0·094
Camembert	45·24	30·31	13·96	5·79	3·69	1·01	7·98	0·067	0·142	0·070
Port du Salut	47·72	24·96	19·92	3·50	1·73	2·12	4·27	0·002	0·535	0·235
Crescenza	56·75	31·34	15·63	3·28	1·34	1·56	6·65	0·000	0·000	0·020

Hard Cheese (*Duclaux*)

	Water	Fat	Insoluble protein	Soluble protein	Salt	Ash	Caseone	Ammonia		Butyric acid
								Free	Combined	
Gorgonzola	40·75	31·88	12·00	11·96	2·42	1·99	10·11	0·020	0·036	0·125
Dutch	37·31	24·41	22·09	10·41	2·84	2·85	8·44	0·001	0·270	0·212
Gruyère	36·00	29·29	24·54	6·30	0·57	3·30	4·33	0·029	0·058	0·250
Parmesan	30·09	26·04	23·70	14·72	1·76	3·69	15·80	0·003	0·250	0·180
Grana di Reggio	32·56	21·75	25·56	16·71	1·65	3·42	18·50	0·002	0·150	0·200

Metals in cheese

The presence of copper has been noted in cheese by Besana; it is derived from the use of copper vessels. Stoddart has found metallic lead in Canadian cheese; its presence appeared to be accidental. Many workers have drawn attention to the presence of metals, mainly due to the modern method of wrapping cheese in metal foil (cf. Manley (1930), Davies (1932a), Elton (1929), Dyer and Taylor (1931); see also Chapter 25).

Fat and water in cheese

Pien and Maurice (1938) examined 50 Camembert cheeses from the same factory after varying periods of from 2 days to 13 months. The amount of water decreased over this period from 53 to 15 per cent, whilst the fat increased from 22·4 to 43·4 per cent. The lactose and lactic acid decreased from 4·4 per cent to nil in less than 2 months. At the end of the period the proportion of soluble nitrogen had reached 41·5 per cent of the total nitrogen, by which time the ammoniacal nitrogen was 60 per cent of the soluble nitrogen.

Partridge (1932) found the following acidities in samples of Gorgonzola and Stilton cheese as bought (Table 12.11).

Table 12.11—Composition of Gorgonzola and Stilton cheese

Number	Water per cent	Acidity as lactic acid per cent	Number	Water per cent	Acidity as lactic acid per cent
1	28·1	0·36	11	35·5	1·12
2	34·6	0·54	12	32·9	1·22
3	—	0·55	13	32·0	1·29
4	35·6	0·73	14	44·9	1·42
5	36·1	0·77	15	32·3	1·44
6	44·3	0·85	16	42·5	1·48
7	28·8	0·89	17	37·5	1·60
8	29·3	0·90	18	38·5	1·72
9	38·2	0·91	19	27·9	1·84
10	31·7	1·05	20	27·5	2·14

Sajous (1930) has published results for the amount of fat in Gruyère cheese. His results are given as percentage of fat on the dry matter. Eleven samples contained from 40·2 to 53·1 per cent with a mean of 46·1 per cent. Thirty-seven other samples were examined with the following results—

4 contained over 50 per cent of fat;
23 contained from 45 to 50 per cent of fat;
9 contained from 40 to 45 per cent of fat;
1 contained 38·4 per cent of fat.

The results obtained on the analysis of a considerable number of samples purchased under the Food and Drugs Acts are given in Tables 12.12 to 12.14. The samples were examined in the Lancashire County Laboratory.

Table 12.12—Water in cheese 1908 to 1936

Per cent water	Years		
	1908–1930	1931–1935	1936
22·1 to 23·0	3	1	0
23·1 ,, 24·0	5	2	1
24·1 ,, 25·0	5	3	0
25·1 ,, 26·0	6	0	0
26·1 ,, 27·0	7	2	1
27·1 ,, 28·0	14	8	1
28·1 ,, 29·0	24	6	0
29·1 ,, 30·0	38	8	1
30·1 ,, 31·0	44	13	0
31·1 ,, 32·0	65	22	2
32·1 ,, 33·0	59	29	0
33·1 ,, 34·0	71	24	1
34·1 ,, 35·0	72	17	3
35·1 ,, 36·0	82	29	2
36·1 ,, 37·0	88	33	4
37·1 ,, 38·0	96	38	7
38·1 ,, 39·0	114	40	2
39·1 ,, 40·0	115	45	8
40·1 ,, 41·0	124	42	10
41·1 ,, 42·0	111	50	10
42·1 ,, 43·0	100	34	10
43·1 ,, 44·0	57	22	16
44·1 ,, 45·0	36	9	10
45·1 ,, 46·0	29	6	1
46·1 ,, 47·0	24	2	4
47·1 ,, 48·0	10	0	1
48·1 ,, 49·0	10	1	0
49·1 ,, 50·0	4	0	0
50·1 ,, 51·0	4	0	0
51·1 ,, 52·0	3	0	0
52·1 ,, 53·0	5	0	0
53·1 ,, 54·0	4	0	0
54·1 ,, 55·0	9	0	0
55·1 ,, 56·0	4	1	0
Over 56·0	11	1	0
TOTALS . .	1453	488	95

Table 12.13—Fat in dry matter of cheese, 1908 to 1936

Per cent of fat in dry matter	Years		
	1908–1930	1931–1935	1936
20·1 to 21·0	3	—	—
21·1 ,, 22·0	2	—	—
22·1 ,, 23·0	0	—	—
23·1 ,, 24·0	1	—	—
24·1 ,, 25·0	1	—	—
25·1 ,, 26·0	4	—	—
26·1 ,, 27·0	3	—	—
27·1 ,, 28·0	0	—	—
28·1 ,, 29·0	3	1	—
29·1 ,, 30·0	9	1	—
30·1 ,, 31·0	6	—	—
31·1 ,, 32·0	10	—	—
32·1 ,, 33·0	10	—	—
33·1 ,, 34·0	8	—	—
34·1 ,, 35·0	4	—	—
35·1 ,, 36·0	5	—	—
36·1 ,, 37·0	5	—	—
37·1 ,, 38·0	2	—	—
38·1 ,, 39·0	3	—	—
39·1 ,, 40·0	4	—	—
40·1 ,, 41·0	9	—	—
41·1 ,, 42·0	7	2	—
42·1 ,, 43·0	21	1	—
43·1 ,, 44·0	18	3	—
44·1 ,, 45·0	42	15	—
45·1 ,, 46·0	61	16	3
46·1 ,, 47·0	88	26	6
47·1 ,, 48·0	93	33	7
48·1 ,, 49·0	129	48	9
49·1 ,, 50·0	153	54	16
50·1 ,, 51·0	148	68	13
51·1 ,, 52·0	157	61	15
52·1 ,, 53·0	135	47	8
53·1 ,, 54·0	108	42	3
54·1 ,, 55·0	79	27	5
55·1 ,, 56·0	46	17	3
56·1 ,, 57·0	38	10	4
57·1 ,, 58·0	12	8	2
58·1 ,, 59·0	5	4	1
59·1 ,, 60·0	2	2	—
60·1 ,, 61·0	5	1	—
61·1 ,, 62·0	3	0	—
65·1 ,, 66·0	0	1	—
TOTALS . .	1442	488	95

Table 12.14—Cheese: fat in dry matter, 1908 to 1936

Type of cheese	No. of samples	Average fat per cent	Extremes per cent
Colonial	26	52·7	29·2 to 59·6
Processed	21	50·2	45·7 ,, 54·9
Cheshire	192	51·0	41·1 ,, 58·2
Gorgonzola	14	50·2	41·0 ,, 55·5
Cheddar	15	50·2	44·0 ,, 57·6
Lancashire	280	49·7	42·1 ,, 57·8
Soft	13	46·3	2·8 ,, 57·4
Dutch	9	33·3	29·8 ,, 41·0

The fats obtained from the above samples were examined in the butyro-refractometer from the years 1931 to 1936 inclusive. The results obtained are given in Table 12.15—

Table 12.15—Refractions of cheese fats in years 1931 to 1936

Refraction at 40° C	Year						
	1931	1932	1933	1934	1935	1936	Total
40·0 to 40·3	—	—	1	1	—	—	2
40·4 ,, 40·7	3	1	—	1	—	1	6
40·8 ,, 41·1	1	—	—	—	—	—	1
41·2 ,, 41·5	2	2	3	6	2	3	18
41·6 ,, 41·9	2	1	1	2	3	5	14
42·0 ,, 42·3	5	6	6	10	15	12	54
42·4 ,, 42·7	5	8	8	12	13	15	61
42·8 ,, 43·1	10	10	12	9	11	15	67
43·2 ,, 43·5	12	17	12	15	27	14	97
43·6 ,, 43·9	12	17	20	22	17	10	98
44·0 ,, 44·3	15	11	12	12	19	15	84
44·4 ,, 44·7	15	7	12	5	5	5	49
44·8 ,, 45·1	10	2	2	3	4	—	21
45·2 ,, 45·5	2	1	—	1	1	—	5
45·6 ,, 45·9	1	—	—	—	—	—	1
46·4 ,, 46·9	1	—	—	—	—	—	1
Number of samples	96	83	89	99	117	95	579
Average refraction	43·66	43·39	43·65	43·21	43·28	43·06	43·34

In Table 12.16 the results obtained in Table 12.15 have been classified in accordance with the month in which the sample was purchased. With these cheese fats there is not an exactly corresponding cycle in the value of the refractive index, as with butter fat. With butter fat, the maximum average for the refractive index occurs in September, and the minimum in December or January. With cheese fat the maximum occurs in December and the minimum in May. The remarks made under butter fat on p. 239 will have at least an equal application to cheese.

Table 12.16—Refractions of cheese fats in months, 1931–1936

Refraction at 40° C	Months												Tota
	Jan.	Feb.	Mar.	Apl.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
40·0 to 40·3	—	—	—	—	—	—	—	1	—	—	1	—	2
40·4 ,, 40·7	—	—	—	2	1	2	—	—	1	—	—	—	6
40·8 ,, 41·1	—	—	—	—	1	—	—	—	—	—	—	—	1
41·2 ,, 41·5	—	1	2	4	6	2	—	3	—	—	—	—	18
41·6 ,, 41·9	—	1	3	4	1	2	1	—	—	—	2	—	14
42·0 ,, 42·3	6	6	5	12	10	6	1	2	1	2	3	—	54
42·4 ,, 42·7	9	12	3	8	8	8	1	3	4	1	3	1	61
42·8 ,, 43·1	9	9	6	11	4	4	7	3	2	7	4	1	67
43·2 ,, 43·5	9	8	4	12	6	10	9	6	7	11	8	7	97
43·6 ,, 43·9	13	9	3	3	3	11	10	7	4	9	17	9	98
44·0 ,, 44·3	15	2	5	1	1	3	1	8	14	12	14	8	84
44·4 ,, 44·7	8	1	2	3	1	2	6	2	5	7	7	5	49
44·8 ,, 45·1	4	2	—	3	1	1	2	—	1	3	4	—	21
45·2 ,, 45·5	—	1	1	—	—	—	—	—	1	1	1	—	5
45·6 ,, 45·9	—	1	—	—	—	—	—	—	—	—	—	—	1
46·4 ,, 46·9	—	—	1	—	—	—	—	—	—	—	—	—	1
No. of samples	73	53	35	63	43	51	38	35	40	53	64	31	579
Average refraction	43·56	43·19	43·17	42·81	42·57	43·02	43·59	43·25	43·60	43·76	43·70	43·82	43·3

An important paper has been published by Nicholls (1941) on water and fat in English cheese. A total of 2,140 samples was examined, these being representative of English cheese of pre-war period, some being factory cheese and some farmhouse cheese. The average results obtained are given in Table 12.17.

Nicholls deduced a general equation of the type $W + mF = K$, or, if the cheese has been salted,

$$W + mF = \frac{100 - S}{100} K,$$

where W = percentage of water, F percentage of fat, and S the percentage of salt remaining in the cheese. The constant K is 93·3 for cream cheese and 93·0 for pressed cheese. The factor m is dependent upon the fat content of the original milk or cream. Tables are given for the relationship between the factor m and the fat in the original milk or cream, less the fat in the whey.

Table 12.17—Water and fat in English cheese (*Nicholls*)

Type	No. of samples	Water per cent	Fat per cent	Fat in dry matter per cent
Cheshire	606	40·53	29·96	50·3
Cheddar	363	36·69	31·82	50·2
Lancashire	403	44·28	27·39	49·1
Stilton (White) . .	124	41·76	32·44	55·37
Stilton (Blue)	212	37·64	35·71	57·2
Caerphilly	234	44·67	28·43	51·23
Wensleydale	111	45·03	27·72	50·23
Leicester	24	39·79	30·21	50·0
Derby	11	39·95	30·59	50·7
Gloucester	3	41·0	28·8	49·0
Cream	49	24·5	71·6	—

By taking an average value of 0·3 per cent or a maximum value of 0·6 per cent for the whey fat, an average value or a maximum value for the fat in the original milk or cream can be obtained. The factor m is regarded as a slightly better criterion than the percentage of fat in the dry substance, for determining whether or not a sample has been made from whole milk.

Recent (1947 to 1950) analytical data for cheese in England are given in Tables 12.18 to 12.21. It will be observed that Cheshire cheese is more variable both as regards moisture and fat content in dry matter than Cheddar. It may be emphasised that these samples were taken by the graders who may have " weighted " their sampling slightly by concentrating on the cheese likely to be deficient in fat. Cheddar cheese is graded at 4 to 8 weeks and Cheshire at 2 to 4 weeks.

" Danish blue " has become a popular variety in England and is now imported from Denmark, Holland and Germany. It is of the Roquefort type and contains from 41 to 44·5 per cent moisture and from 31·5 to 35 per cent fat.

Table 12.18 shows the distribution of moisture content in random groups of 100 samples of cheese of various descriptions made in Britain, and also in a group of imported cheese. As regards the imported cheese the varieties sampled are not known. Table 12.19 gives a similar distribution for fat as a percentage of dry matter.

Table 12.18—Moisture content (per cent) of British and imported cheese

Moisture	Cheshire	Lancashire	Cheddar	Wensleydale	Imported
12					1
13					1
26					1
27		1			2
28					1
29					2
30					1
31					2
32			2		6
33			1		7
34	1		3		4
35	3		6	1	9
36	2		15	1	9
37	1		19		4
38	4	1	16	1	4
39	5	2	19	5	2
40	12	4	12	5	5
41	16	6	4	6	4
42	12	10	2	18	1
43	13	9		10	3
44	12	18		10	5
45	7	18		12	6
46	7	17		7	2
47	1	7		7	1
48	2	3		7	2
49	2	4		3	—
50				2	1
51				1	1
52				2	2
53				—	2
54				1	—
55				—	2
56				1	—
57					1
58					—
59					2
60					—
61					3
66					1
	100	100	100	100	100

Table 12.19—Fat as per cent of dry matter in British and imported cheese

Fat/ dry matter × 100	Cheshire	Lancashire	Cheddar	Wensleydale	Imported various kinds
14					1
39					1
40					1
43					2
44			1		1
45		1			2
46		2	1	1	3
47	1	2	4		
48	1	4		2	1
49	1	8	7	1	3
50	8	4	7	1	2
51	6	4	10	3	6
52	10	5	15	5	5
53	6	8	10	2	10
54	6	9	12	17	5
55	6	10	6	4	5
56	4	11	8	4	8
57	10	8	6	1	4
58	10	6	6	11	7
59	6	5	3	10	5
60	5	3	2	7	7
61	6	3		7	3
62	2	2	1	4	8
63	3	2		3	3
64	2	3	1	5	1
65				4	2
66	1			3	1
67				2	1
68	3				2
69				3	
	100	100	100	100	100

Table 12.20—English Cheddar—creamery cheese: three-year period, October 1947 to September 1950

Water per cent	FAT IN MOISTURE-FREE SUBSTANCE, PER CENT																								TOTALS
	42–	43–	44–	45–	46–	47–	48–	49–	50–	51–	52–	53–	54–	55–	56–	57–	58–	59–	60–	61–	62–	63–	64–	65–	
45–																	1								4
44–															1										1
43–											1	1	2	1				1							6
42–											1		1	1	1				1		1				6
41–								1	1		3	2		4		1					1	1			14
40–				1			3		2	2	1	4	2	2	1	4	2	1	1	1				1	28
39–					2		1	3	5	1	2	3	4	6	1							1			29
38–	1			2	3	3	4	4	7	5	4	8	10	10	2	2	1	2	1						69
37–		1		1	1	3	7	12	5	8	4	16	15	10	8	5	4	1						1	102
36–			2		2	5	8	7	14	8	7	3	4	5	4	2	2	1							74
35–				1	2	5	7	10	10	3	3	6	4	3	1					1					56
34–						3	7	6	4	2	3	3	1				1								30
33–			1		1			2	1	1	1	2			1										10
32–					1			1	2																4
31–				1				1									1								3
30–												1		1											2
29–				1																					1
TOTALS ..	1	1	3	7	12	19	37	47	51	30	30	49	43	43	20	14	12	6	3	2	2	2	—	2	436

Table 12.21—Cheshire—creamery cheese: three-year period, October 1947 to September 1950

Water per cent	FAT IN MOISTURE-FREE SUBSTANCE, PER CENT																									Totals
	44–	45–	46–	47–	48–	49–	50–	51–	52–	53–	54–	55–	56–	57–	58–	59–	60–	61–	62–	63–	64–	65–	66–	67–	68–	
50–							1			*52/1	*51/1															3
49–														1	1			1	1					1		5
48–																	1		1							2
47–									3	1			1				1		1				1			8
46–						2			2	3		2		1	1		1	1	1			3				17
45–						1		1	2	2	3	3	3	2	3	4	2	1			1		1		1	30
44–						1	3	4	6	3	7	6	2	1	2	5	2	1	2							45
43–	1	1		1	2	2	4	3	7	4	7	8	8	8	6	2	3	6	1	1	1					76
42–		1	1		4	2	3	8	8	10	5	7	4	7	5	6	2	2	2	1	1		1			80
41–	1		1	2	4	5	7	4	14	10	10	14	13	5	5	2	3	1	3	1		1				106
40–	1		3	5	5	5	5	12	10	11	6	9	8	4	7	2	2									95
39–				4	2	5	6	10	5	3	9	1	3	3	2	1	2	1				1				58
38–		1		2		2	7	4	3	9	2	4	4	1	2	4		1								46
37–						1	1	1	3	2	2	2	2	2	1											17
36–					3	1	1	2	1		2	1	1	1												13
35–	1					1	1		2		1															6
34–					1			1	1																	3
33–					1	1																				2
32–																										—
31–																										—
30–																										—
29–							1	1																		2
28–						1																				1
Totals . .	4	3	5	14	22	30	40	51	67	59	55	57	49	36	35	26	19	15	12	3	3	5	3	1	1	615

* Water per cent of isolated samples.

Hiscox (1950) has reported some interesting analyses for seven-year-old Cheddar cheese. As might be expected, the degree of degradation of protein and fat was higher than that for cheese of normal age, but the latter was not as high as might have been expected.

Composition of curd and whey

The following table shows the distribution of the various constituents of milk when made into whey and curd—

Table 12.22

	Milk per cent	Per cent in whey	Per cent in curd
Water	87·30	80·80	6·50
Fat	3·75	0·25	3·50
Milk-sugar	4·70	4·40	0·30
Casein	3·00	0·40	2·60
Albumin	0·40	0·40	trace
Ash	0·75	0·60	0·15

The following is the percentage composition of whey according to various authorities—

Table 12.23—Composition of whey (per cent)

	Fleischmann	König (average)	Smetham	Vieth (from skim-milk)
Water	93·15	93·38	93·33	93·00
Fat	0·35	0·32	0·24	0·09
Milk-sugar	4·90	4·79	5·06	5·45
Protein	1·00	0·86	0·88	0·92
Ash	0·60	0·65	0·49	0·54

Richmond found the fat in whey to vary from 0·04 per cent (from skim-milk) to 1·35 per cent, and the solids-not-fat to lie between 6 and 7 per cent, averaging 6·6 per cent, which contain

	Per cent
Milk-sugar	5·08
Protein	0·92
Ash	0·60

On adding an acid to whey, a slight protein precipitate which is difficult to filter is obtained. On heating the acid whey, a soft curd of slight consistency is formed; this substance is a commercial article on the Continent. The composition of the whey and the precipitated curd, after acidifying and boiling, are given as follows (per cent)—

Table 12.24

	By Fleischmann		By Bochicchio
	Whey	Precipitate	Precipitate
Water	93·31	68·5	68·47
Fat	0·10	3·1	5·22
Milk-sugar	5·85	3·2	3·97
Lactic acid	—	0·8	—
Protein	0·27	22·1	18·72
Ash	0·47	2·3	3·62

On boiling whey without acidifying, a precipitate of a similar nature also occurs; this appears to consist of coagulated albumin.

Cheese is sometimes prepared by allowing the milk to become sour spontaneously, salting and pressing the curd, and allowing it to ripen. This variety of cheese is not considered of such good quality as rennet cheese.

Fleischmann stated the composition of the whey thus obtained to be as follows—

	Per cent
Water	93·13
Fat	0·12
Milk-sugar	4·38
Protein precipitated by acetic acid	0·47
,, ,, ,, tannin	0·59
Ash	0·82
Difference (lactic acid ?)	0·49

Vieth showed that whey prepared in this manner undergoes alcoholic fermentation much more readily than rennet whey.

Waite (1941) has discussed chemical and technological aspects of the roller drying of cheese and acid casein whey. Fresh whey can be dried up to 0·22 per cent " lactic acid ". Neutralisation is best by $Ca(OH)_2$ but over-neutralisation produces a dark-coloured inedible product. Acetic-acid casein whey dried without trouble even at acidities as high as 0·40 per cent, and there was considerable loss of acetic acid on the rollers.

Corrections for hydrometer measurements on whey at different temperatures have been published by Roeder (1948b).

A review of the utilisation of whey has been published by Webb and Whittier (1948) and an interesting historical account of the applications of whey has been given by Funck (1948).

Dried whey

The following figures were recently obtained from material of Dutch origin. The acidity is the acidity titratable to phenolphthalein calculated as lactic acid.

Table 12.25

Moisture	4·78	4·20	4·05	4·52	3·80	5·58
Protein (N × 6·38)	11·49	11·71	11·13	11·44	10·76	12·43
Ash	9·45	8·48	8·64	8·46	8·35	8·50
Acid as lactic ..	1·80	1·80	1·90	1·90	1·90	2·80
Lactose (by diff.) ..	72·48	73·81	74·28	73·68	75·19	70·69

OTHER PRODUCTS FORMED FROM MILK BY THE ACTION OF MICRO-ORGANISMS

Besides butter and cheese, in the manufacture of which micro-organisms play an important part, several preparations are made from milk; among these may be mentioned koumiss, kephir, mazoum, and yogurt.

Koumiss

This preparation was originally made from mares' milk by the Tartars. It is a product of combined alcoholic, lactic, and protein-hydrolytic fermentations of milk. Its production first from mares' milk is probably due to the fact that the sugar of this milk very easily undergoes alcoholic fermentation.

The following analyses of mares' milk koumiss are by Vieth—

Table 12.26—Composition of koumiss (per cent)

	1 day old	8 days old	22 days old
Water	91·43	92·12	92·07
Alcohol	2·67	2·93	2·98
Lactic acid	0·77	1·08	1·27
Sugar	1·63	0·50	0·23
Casein	0·77	0·85	0·83
Albumin	0·25	0·27	0·24
Proteoses	0·98	0·76	0·77
Fat	1·16	1·12	1·30
Ash	0·35	0·35	0·35

Koumiss is now very largely made from cows' milk by the selection of special organisms.

Analyses of various kinds of koumiss have been made by Vieth on the preparations of the Aylesbury Dairy Company (see Table 12.27). The carbonic acid present has not been taken into account; it is, however, always present (except in the earlier stages) in sufficient amount to render the koumiss highly effervescent; hence, the preparation has been termed "milk champagne" on this account. The term "casein" in the table includes meta-caseins.

Table 12.27—Composition of koumiss

	1 day old	8 days old	22 days old
	Per cent	Per cent	Per cent
FULL KOUMISS			
Water	88·90	90·35	90·57
Alcohol	0·15	0·94	1·04
Fat	1·35	1·36	1·38
Casein	2·01	1·96	1·88
Albumin	0·30	0·23	0·20
Proteoses	0·34	0·53	0·77
Lactic acid	0·34	0·96	1·40
Sugar	6·03	3·10	2·18
Ash, soluble	0·17	0·23	0·23
,, insoluble	0·41	0·34	0·35
WHEY KOUMISS			
Water	89·74	90·63	91·07
Alcohol	0·30	1·03	1·38
Fat	0·11	0·13	0·15
Casein	0·15	0·14	0·11
Albumin	0·39	0·36	0·32
Proteoses	0·44	0·49	0·58
Lactic acid	0·60	0·91	1·26
Sugar	7·48	5·52	4·34
Ash, soluble	0·37	0·37	0·37
,, insoluble	0·42	0·42	0·42
MEDIUM KOUMISS			
Water	87·55	88·39	88·62
Alcohol	0·29	0·97	1·05
Fat	1·54	1·56	1·58
Casein	1·46	1·40	1·30
Albumin	0·43	0·25	0·14
Proteoses	0·48	0·76	0·97
Lactic acid	0·68	1·20	1·67
Sugar	6·80	4·70	3·90
Ash, soluble	0·28	0·32	0·33
,, insoluble	0·49	0·45	0·44

Table 12.27—Composition of koumiss—*continued*

DIABETIC KOUMISS			
	1 day old	8 days old	22 days old
Water	92·24	92·38	92·55
Alcohol	0·28	0·35	0·57
Fat	0·51	0·52	0·51
Casein	2·19	2·13	2·05
Albumin	0·30	0·25	0·18
Proteoses	0·36	0·48	0·65
Lactic acid	0·75	0·86	1·22
Sugar	2·78	2·42	1·64
Ash, soluble	0·22	0·24	0·26
,, insoluble	0·37	0·37	0·37

Wiley gives the following mean composition of koumiss prepared in America—

	Per cent
Water	89·32
Carbon dioxide	0·83
Alcohol	0·76
Lactic acid	0·47
Proteins	2·56
Fat	2·05
Sugar	4·38

A detailed description of the manufacture of koumiss is given by Haldena (1941).

Kephir (kefir)

This is a preparation of a nature similar to koumiss and is produced from milk by means of kephir grains.

The following is the composition of kephir according to various authorities—

Table 12.28—Composition of kephir (per cent)

	König (mean)	Hammarsten	Vieth (an old sample)
Water	91·21	88·915	90·09
Alcohol	0·75	0·720	0·64
Lactic acid	1·02	0·727	0·44
Fat	1·44	3·088	1·82
Sugar	2·41	2·685	1·87
Casein	2·83	2·904	2·90
Albumin	0·36	0·186	0·07
Proteoses	0·30	0·067	0·45
Ash	0·68	0·708	—

Kephir differs from koumiss chiefly in the comparatively small amount of proteoses it contains, showing that although the alcoholic and lactic fermentations have taken place, the protein-hydrolytic fermentation is very weak.

Struve found in kephir grains—

	Per cent
Water	11·21
Fat	3·99
Protein	51·69

Richmond examined a " kephir powder ", which had the following composition—

	Per cent
Water	2·29
Milk-sugar	89·90
Other organic matter	7·42
Ash	1·39

It appeared to be a mixture of milk-sugar with pulverised kephir grains.

Mazoum

This preparation, introduced from Armenia, where it has been made for centuries, has somewhat the appearance of clotted cream; on warming, it separates into a liquid whey and an insoluble curd.

Richmond has determined the following figures—

	Per cent	
Fat	6·27	curd
Casein	2·56	
Ash	0·04	
Organic solids	5·00	whey
Ash	0·77	
Water	85·38	

There was no evidence of proteoses in the whey.

Mazoum appears to have been produced by the lactic fermentation of milk enriched by cream; the sample examined was very fresh and protein-hydrolytic fermentation was not appreciable.

An organism was separated from mazoum which gave colonies rapidly spreading on the surface of gelatine to 1 cm or more in diameter, and which produced a slight putrid smell. This organism, which was a bacillus, slowly peptonised milk without curdling it, and finally transformed it into a semi-transparent liquid jelly.

Yogurt, yoghurt, yoghourt or Bulgarian sour milk

This preparation is a solid or thick gelatinous liquid of pleasant acid taste. It may contain as much as 2½ per cent of lactic acid, and the proteins are hydrolysed to a considerable extent. The milk used for its preparation is sometimes concentrated or reinforced with skim-milk powder. Davis (1952b) has given an account of the manufacture, constitution and therapeutic properties of yogurt. For cultured milks in general see Burke (1938).

RANCIDITY IN BUTTER FAT

The detection of rancidity in fats is primarily one of taste and smell, but the difficulties of making quantitative observations and the varying susceptibilities of different individuals make it highly desirable to rely on chemical tests. The term " rancidity " is sometimes reserved for the change in flavour which is produced by the liberation of free fatty acids, but as this change is often produced by the same agencies or at the same time as others, the name is often loosely applied to denote several different types of change in a fat, the only connection between them being that they all result in the production of flavours which are objectionable to those who are in the habit of eating fresh fat.

The causes which result in rancidity are many and complex, but they may be roughly divided into several classes, such as (*a*) absorption of odours, (*b*) the action of enzymes, (*c*) the action of micro-organisms, and (*d*) atmospheric oxidation. One or all of these may play their part in the production of objectionable flavours, but it must be remembered that the pleasantness or otherwise of a flavour is largely a matter of education of the palate. The normal Western requirement for a butter fat is that the flavour shall be mild, slightly aromatic, and free from acidity, but the Eastern palate requires—or at least is prepared to support—a much harsher flavour, whilst the well-known salted keg butter, so much liked by Welsh farmers, is most distasteful to those who have become accustomed to the flavour of fresh dairy butter.

The characteristic flavour of fresh butter is due to the presence of diacetyl, $CH_3.CO.CO.CH_3$, of which the content may vary from 0·05 parts per 10^6 for a mild-flavoured butter to 1·2 parts per 10^6 for one of full flavour (Davies, 1936b). Butter prepared from " ripened " cream usually has a fuller flavour than that prepared from " fresh " cream, but the keeping quality of the product is inferior.

The determination of acidity can, of course, be carried out readily; the method adopted is that for free fatty acids as described on p. 502. It is usual to express the result either as the acid value or as the percentage of oleic acid. Rancidity due to other changes than acidity is not so easy to determine. Several methods have been suggested, each of which has been the subject of a number of modifications. The better-known tests are the peroxide value, the Kerr modification of the Issoglio method, and the Kreis test—the latter being the most widely used. It cannot be said that, up to now, any one of these tests is quantitative; in fact, more than one Official Committee has stated that none of the colour reactions can be considered as sufficiently reliable to use as a standard method, and that taste and odour remain the chief criteria of rancidity; at the same time, such chemical tests have their uses, as they make it possible to record values (rough though they must be) of the reactions of a particular fat for comparison, at other times, with results obtained from other samples of the same type of fat.

The complex problem of rancidity has been reviewed by Sundberg and Hultberg (1947). These authors have made some very useful suggestions for eliminating errors in methods for the estimation of peroxides, aldehydes and methyl ketones. Rancidity is also associated with increases in acid number and refractive index. No evidence could be obtained that rancidity is associated with polymerisation. Loftus-Hills and Conochie (1945) have shown that traces of magnesium chloride in solid salt can catalyse the oxidation of susceptible fats, and that halide ions and hydrogen ions in the serum, especially over the range pH 6 down to 3, accelerate the reaction. It is suggested that acid halides in the presence of peroxides may yield the free halide.

Krukovsky *et al.* (1948) have used the re-emulsification test (fat in skim milk) to study the effect of ascorbic acid on the oxidative deterioration of milk fat. Destruction or removal of all the vitamin C prevents oxidised flavour, and it appears that dehydro-ascorbic acid may act as a catalyst. The pure fat does not suffer deterioration. Beier (1948) has described a simple test for the deterioration of fats, involving the reduction of acid bichromate by lower fatty acids, aldehydes and ketones.

Dunkley (1951) finds that the rancidity of milk can be estimated better by changes in surface tension than by a determination of fat acidity.

Rancidity was seldom detected in samples when the surface tension was greater than 46 dynes per cm but could be estimated in most samples when the value was below 45 dynes per cm.

Fat rancidity influences both the surface tension of the milk and the surface tension value at which rancidity can be detected organoleptically. The taking and treatment of the sample must be carefully controlled.

The whole subject of rancidity in edible fats has been discussed very fully by Lea (1938).

Tests for rancidity

The estimation of peroxide oxygen. Lea (1938, 1941) determines this in the following way—

One gram of the oil or fat is weighed into a numbered and tared pyrex test-tube, approximately 17 mm in diameter. Powdered potassium iodide (*ca.* 1 gm) is added, followed by 20 ml of a mixture of glacial acetic acid and chloroform or carbon tetrachloride (2 : 1 by volume). A rubber stopper, bored with one hole, is fitted, and nitrogen or carbon dioxide passed into the air-space above the liquid for about one minute to displace most of the air. The tube is then heated in an inclined position over a small flame applied to its lower end, a finger resting lightly over the hole in the stopper and the tube rotating slowly to prevent cracking. As soon as the solution is bubbling fairly freely, the tube is plunged into a boiling water-bath. The liquid boils smoothly, chloroform vapour expelling any remaining traces of air. When vapour begins to issue from the tube, as felt by condensation on the stopper, the finger is removed and a glass plug forced in. This operation is preferably carried out with the liquid frothing nearly to the top of the tube. In the presence of non-fatty material, the tube is now shaken for a few seconds to ensure complete extraction of the fat. Otherwise it is simply cooled under the tap and the contents poured into about 30 ml of water. The tube is rinsed out with a little water, and the free iodine titrated with 0·002 N sodium thiosulphate, adding starch solution as the end-point is approached.

(1 ml of 0·002 N thiosulphate per gram is equivalent to 1 millimole, or

2 milli-equivalents of peroxide, 16 mgm of *active* oxygen or 32 mgm of total peroxide oxygen per kilogram of fat.)

The Kerr modification of the Issoglio method is carried out as follows—

Twenty-five grams of the fat are weighed into a 200 ml Erlenmeyer flask and 100 ml of distilled water added. The flask is allowed to stand on the steam-bath for two hours with occasional shaking, after which the contents are filtered through a wetted filter-paper into a 100 ml graduated flask, cooled, made up to the mark, and mixed thoroughly. To 10 ml of this solution are added 50 ml of water, 10 ml of 20 per cent sulphuric acid, and 50 ml of 0·01 N potassium permanganate, and the mixture is boiled for five minutes under a ground-in reflux condenser. The liquid is then cooled, 50 ml of 0·01 N oxalic acid run in, and the unoxidised excess titrated with 0·01 N permanganate. If T and t represent the volumes of permanganate solution used up in the oxidation and in a blank test, respectively, and W be the weight of fat taken, then the " oxidisability value ", which represents the number of milligrams of oxygen required to oxidise the water-soluble constituents from 100 g fat, is given by

$$\frac{(T - t)\,80}{W}$$

The following modification of the *Kreis test* has been suggested by the American Oil Chemists' Society—

Three grams of oil are dissolved in 6 ml of benzene in a small glass-stoppered cylinder and shaken with 3 ml of concentrated hydrochloric acid for one minute. Five drops (0·1 ml) of a 5 per cent solution of phloroglucinol in alcohol are then added, the mixture shaken for one minute and separated by means of a (hand) centrifuge. Approximately 2 ml of the clear aqueous layer are transferred to a 1 cm glass cell and matched against the colour standards of the Lovibond tintometer (B.D.H. pattern with artificial light attachment). Alternatively, the solution contained in a small test-tube may be matched in a block comparator against the standards, placed in series with a blank solution. By the use of a little yellow or blue in conjunction with the red slides, an accurate match can readily be obtained. Results may be expressed as the sum of two colours, or 1 yellow unit may be considered as the equivalent of 0·2 red. In the case of samples of fat giving colours deeper than about 10 units on the Lovibond scale, the quantity taken must be reduced, and the value obtained corrected to the normal weight. Blank determinations should be carried out without the phloroglucinol, to allow for any charring effect of the acid on the oil, and without the oil to check the purity of the reagents.

CHAPTER 13

ANCILLARY MATERIALS

RENNET, ANNATTO

Annatto

Villela (1944) has shown that bixin is the only carotenoid in annatto seed, so that extracts of the seed are useless as a source of provitamin A. Titov (1941) has recommended the use of 2 parts castor oil, 2 parts mustard seed oil and 1 part of sesame oil as a solvent for annatto.

The use of annatto as an added colouring-matter in milk is discussed in Chapter 18. For a general account see the article on " Annatto " in Davis's *Dictionary of dairying*.

Rennet

(i) *Purification*

Rennin has been crystallised by Berridge (1943) by salt precipitation at pH 5·4 and absorption on alumina. One part of the pure enzyme could clot 10^7 parts of reconstituted milk in 10 min. at 37° C. He concludes that the enzyme is a protease acting on haemoglobin at an optimum pH of 3·7 (Berridge, 1945). The preparation of a purified rennet has been described by Hankinson and Palmer (1942). Hankinson (1943) later prepared crystalline rennin by salting out, dialysis and acid precipitation. It has an isoelectric point at about 4·55 and a compositional analysis of 51·4 per cent C, 7·19 per cent H, 14·51 to 15·05 per cent N, 1·46 per cent S, 0·041 per cent P, 0·0035 per cent Cu and 0·2 per cent ash. It does not contain Fe. Bhima Rao *et al.* (1942) have purified rennin and obtained a preparation free from N, P and S. The activity lost at pH 7 to 7·4 is restored by addition of zinc chloride or boiled enzyme.

(ii) *Mechanism of rennet action*

Moriyama (1941) suggests that rennin is a lipoprotein, and that the precipitation of casein is a denaturation involving a chain reaction. Berridge (1942) has utilised the fact that the second or precipitation stage of rennet action does not occur at low temperatures, to study the stages separately. The temperature coefficient of the second phase is 1·3–1·6 per °C and this phase is probably a denaturation. The logarithm of this reaction is related linearly to temperature.

Hankinson and Palmer (1943) conclude from a study of various physical phenomena in rennet action, that the reduced stability is due to an increased hydration, the associated decrease in electrokinetic potential being of secondary importance.

According to Struble and Sharp (1940) rennin in whey has a zone of maximum stability at 48° C at pH 4, the enzyme being easily destroyed below pH 3·5 and above 4·5. Rennin is inactivated in 14 min. at 50° C at pH 6·8.

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Pyne (1945) has produced more evidence concerning the effect of calcium on rennet coagulation. The increase in clotting time which occurs when heated milk is allowed to stand is due to the loosening of the calcium phosphate from the calcium caseinate. Gyr (1947) finds that increasing concentration of rennet, increasing acidity pH 6·4 down to 5·3, and increasing temperature 40° to 60° C all increased the *rate* of contraction but not the final value. Addition or removal of calcium delayed contraction. A useful review of theories of rennet action has been compiled by Beau (1941).

(iii) *Preparation*

Berridge *et al.* (1943) have evolved a method of obtaining abomasal juice by a fistula method. The rennet could be used to make normal cheese. Fomin (1941) has also described the successful use of this method.

(iv) *Tests for rennet strength*

The testing of rennet strength is not yet put on a satisfactory scientific basis, as it is not possible to prepare either a standard rennet or a standard substrate. Mulder and Radema (1947) have found that when the pepsin activities of rennet preparations vary widely, the former are not a reliable index of rennet strength.

Tarassuk and Richardson (1941b), have shown that the use of *raw* skim milk for the reconstitution of milk powder leads to low pH values, surface tensions and lack of clotting ability with rennet, due to lipolysis. Addition of the *higher* fatty acids to milk delays or inhibits rennet coagulation.

An apparatus for measuring the firmness of rennet curd in terms of shear modulus has been described by Rowland and Soulides (1942). According to the results obtained, fat does not affect curd firmness, and lactic acid increases it up to a maximum of pH 6·2. The effect of $CaCl_2$ for normal milks can be ascribed to the lowering of pH, but for soft curd milks the Ca ion has a specific effect in addition to the pH effect. A 25 per cent addition of soft curd milk prevented proper curd formation by ordinary milk. Increasing rennet concentrations gave increasingly firm curds. King and Melville (1939 and 1940) have described a viscometer for measuring rennet strength, and Berridge (1952) has used skim milk powder reconstituted in 0·01 M $CaCl_2$ as a standard substrate.

(v) *Vegetable rennets*

According to Krishnamurti and Subrahmanyan (1948) the edible fig (*Ficus carica*) gives the strongest rennet extract of the plants tested. It behaves like animal rennets and is inactivated above 55° C. They have also described the preparation of a rennet from *Streblus asper*, and claim to have made satisfactory cheese with fig rennet. Dastur *et al.* (1948) have prepared a rennet from *Withania coagulans* by alcohol or acetone precipitation. Cheeses prepared with this were satisfactory except for a slight bitterness in the hard cheese. Yeshoda (1941) prepared rennet from *Withania coagulans* by precipitation by ammonium sulphate and acetone. The optimum temperature was 48° (see below) and the preparation was not proteolytic towards gelatine. Narain and Singh (1943) found the best source of vegetable rennet to be the berries of *Withania coagulans*. Alcohol precipitation yielded 1·9 g powder from 100 g berries which clotted

95 lb milk of acidity 0·21 per cent in 45 min. No differences could be detected in cheese made with this rennet. The enzyme had an optimum temperature of 75° C, against 41° for calf rennet, but otherwise behaved similarly to animal rennets. Kothavalla and Khubchandani (1942) claim to have made Cheddar and soft cheese successfully with rennet from *Withania coagulans*.

For a general account of rennet see the articles on " Rennet " and " Cheese " in Davis's *Dictionary of dairying*.

CHAPTER 14

LEGISLATION ON THE PRODUCTION AND SALE OF DAIRY PRODUCTS IN ENGLAND AND WALES

The various Acts and Regulations concerned with the sale of dairy products in this country which have been issued since 1938 have all been included in one general consolidation Act, which is now referred to as " The Food and Drugs Act, 1938 to 1950 ". This major Act thus includes the following Regulations and Orders:

The Sale of Milk Regulations, 1939.
The Public Health (Condensed Milk) Regulations, 1923 and 1927.
The Public Health (Dried Milk) Regulations, 1923 and 1927.
The Milk (Special Designations) Order, 1936 and 1949.
The Food and Drugs (Milk and Dairies) Act, 1944.
The Milk and Dairies Regulations, 1949 (Stat. Instr. No. 1588).
The Milk (Special Designations) (Raw Milk) Regulations, 1949 (Stat. Instr. No. 1590.)
The Milk (Special Designations) (Pasteurised and Sterilised Milk) Regulations, 1949 (Stat. Instr. 1589.)
The Food and Drugs (Milk, Dairies and Artificial Cream) Act, 1950. (This is mainly the consolidation of Part 2 of the 1938 Act.)

It should be noted that the only legal chemical standards which apply to milk generally are those laid down in the 1939 Regulations, which define presumptive minimum standards of 3·0 per cent fat and 8·5 per cent solids-not-fat. These apply to all milks whether designated or not. For Channel Island (sometimes referred to as " Jersey ") and South Devon milk a standard of 4·0 per cent fat is required. This was laid down by S.R. and O. 1947, No. 2032, but is really a condition of contract and failure to satisfy the standard is not legally an offence. " Separated milk " now includes skimmed milk, and there is a presumptive legal minimum standard of 8·7 per cent solids-not-fat.

There are no bacteriological standards for ordinary milk, but the 1949 Regulations recognised four types of designated milks—two raw milks (Accredited and Tuberculin Tested) and two heated milks (sometimes called heat-treated), Pasteurised and Sterilised. Accredited milk will cease to exist after 1st October, 1954. The raw Designated Milks must comply with the $4\frac{1}{2}/5\frac{1}{2}$ hour methylene blue test, the plate count and coli tests formerly applying to these being now not required. Pasteurised milk has to comply with the phosphatase test for efficiency of pasteurisation and a half-hour methylene blue test as a measure of keeping quality. Sterilised milk must satisfy a turbidity test for efficiency of sterilisation.

The Food and Drugs Act, 1938

This is an extremely comprehensive enactment which aims at consolidating, with amendments, previous legislation relating, in its broadest sense, to food. Some idea of its scope will be gauged from the fact that it embodies provisions from some 29 Acts dating from the 16th century; in addition to dairy produce it is concerned with food and drugs generally, and contains provisions with regard to markets, slaughter-houses and knackers' yards.

The sections with which dairy chemists are chiefly concerned are: Sections 1 to 8, 14, 16, 20 to 29, 32 to 36, 37, 68 to 71, 73, 100 and the 1st and 3rd Schedules of the Act. Briefly the contents of these are as follows.

Sections 1 to 8 contain the main principles governing the sale of foodstuffs, including legislation forbidding the addition of injurious substances to foods, restricting the abstraction of constituents from food, and prohibiting the sale of an article other than the one demanded by the purchaser. In addition, these sections contain provisions as to the labelling of foodstuffs and also give the Minister of Health power to make regulations governing the preparation, sale, etc., of foodstuffs other than milk.

Sections 14, 16 and 37 concern ice-cream and deal with such questions as the registration of premises, the display of the vendor's name and address on carts, etc., and the notification of any milk-borne disease among the staff (see Part II of the 1st Schedule).

Sections 20 to 26 deal specifically with milk and dairies and give the Minister of Health wide powers to make " Milk and Dairies Regulations " for any of the following purposes: registration of dairies, etc.; inspection of dairies, staff and utensils; general sanitation of dairies and dairy appliances; cleanliness of milk from bacteriological and chemical standpoints; cooling, labelling and distribution, etc., of milk; and prescribing the conditions under which " special designation " milks may be produced or sold. Power is also given to the Minister of Agriculture and Fisheries to make regulations stating the presumptive standards for the normal constituents of milk.

Section 24 prohibits the addition of any of the following substances to milk: water, colouring-matter, dried or condensed milk or liquid reconstituted therefrom, separated milk, or a mixture of cream and separated milk. This section also forbids the sale under the designation of milk of any liquid in the making of which separated milk, or dried or condensed milk has been used. It is interesting to note that while this section does not specifically prohibit the addition of preservatives to milk, these are automatically prohibited under Sections 1 and 7 read in conjunction with the Public Health (Preservatives, etc. in Food) Regulations, 1925 to 1940.

Section 25 prohibits the sale of tuberculous milk or the milk of cows suffering from the diseases specified in Part I of the 1st Schedule.

Sections 27, 28 and 29 refer to artificial cream, which is defined later in the Act as meaning " an article of food which, though not cream, resembles cream and contains no ingredient which is not derived from milk except water or any substance which may lawfully be contained in an article sold as cream, being some substance not injurious to health which in the case of cream may be required for its production or preparation as an article of commerce in a state fit for carriage or consumption, and which has not been added fraudulently to increase bulk, weight or measure or conceal inferior quality ". It appears to the revisers of this volume that this product would be better described under the term " reconstituted cream ", which is more appropriate to a product prepared from the constituents of milk by the addition of water. True " synthetic " cream, i.e. cream made from fats other than butter fat, is very liable to be confused in the lay mind with the artificial cream of the Act.

Sections 32 to 36 deal with margarine, margarine cheese, butter and milk-blended butter. It is an offence to sell any butter or any margarine which contains more than 16 per cent of moisture; any milk-blended butter which contains more than 24 per cent of water; or any margarine which contains more than 10 per cent of butter fat. Furthermore, any label or advertisement stating that margarine contains butter shall also declare the percentage of butter present, and this statement is required not to differ by more than two from the actual percentage.

Conditions for the labelling of margarine, margarine-cheese and milk-blended butter are also included. For margarine these are briefly as follows. (1) Every container of margarine shall be durably marked on all sides with the word " margarine " in block letters not less than $\frac{3}{4}$ inch long. (2) Every parcel exposed for retail sale shall bear the word " margarine " in block letters not less than $1\frac{1}{2}$ inches long. (3) Margarine sold by retail, if not marked as above, shall be delivered in an outer wrapper which bears only the word " margarine " in block letters not less than $\frac{1}{2}$ inch long. The only other wording allowed is a declaration of the weight of the contents.[1] (4) Margarine shall not be described in any label or advertisement by any name other than " margarine " or a name combining the word " margarine " with an *approved* fancy name, which must be in lettering of the same colouring and in type not larger than the word " margarine ". No fancy name can be approved by the Minister of Agriculture and Fisheries which is in any way suggestive of butter or anything connected with the dairy interest.

These sections also include provisions for the registration of factories and wholesale premises dealing in margarine, margarine-cheese, or milk-blended butter; the registration of butter factories; and the keeping of a register of all consignments of margarine, margarine-cheese and milk-blended butter.

It is an offence to have any oil or fat capable of being used as an adulterant of butter in any butter factory.

Sections 68 to 71 refer to sampling and the following points should be noted. A sampling officer under this Act can take (1) samples of any butter or cheese, or substances resembling butter or cheese, which are exposed for sale and not marked as margarine, margarine-cheese or milk-blended butter; (2) samples of any food, or substances used in the preparation of food, found on premises which he has entered in the execution of his duty; (3) samples of milk at any dairy, at any time while it is in transit, or at the place of delivery to the consignee, etc.; (4) samples of margarine, margarine-cheese or milk-blended butter from containers forwarded by public conveyance.

A sampling officer or other person taking or purchasing a sample with the intention of submitting it to be analysed by a Public Analyst, should, as soon as the sample has been procured, inform the seller or his agent of his intention of having the sample analysed, and he should then and there divide it into three parts, mark, seal or fasten up each part and should (1) deliver one part to the seller or his agent, either by hand or by registered post; (2) retain one part for future comparison, and (3) if he thinks fit to have an analysis made, submit one part to the public analyst.

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[1] Under the Emergency Powers (Defence) General Regulations, an order (S.R. & O. 1940, No. 698) was made, amending the labelling requirements of margarine, the effect of which was to permit other wording on the outer wrapper, which should not appear, however, in bigger print than the word " margarine ".

Section 73 empowers authorised officers of the Minister of Agriculture and Fisheries to enter and inspect and take samples on premises registered in connection with the manufacture, etc., of butter, margarine, margarine-cheese or milk-blended butter.

Section 100 deals with definitions. Butter is defined as " the substance usually known as butter, made exclusively from milk, with or without salt or other preservative, and with or without the addition of colouring-matter ". Cheese as " the substance usually known as cheese, containing no fat other than fat derived from milk ". Cream as " that part of milk rich in fat which has been separated by skimming or otherwise ". Margarine as " any food, whether mixed with butter or not, which resembles butter and is not milk-blended butter ". Margarine-cheese as " any substance prepared in imitation of cheese and containing fat not derived from milk ". Milk-blended butter as " any mixture produced by mixing or blending butter with milk ". No definition of milk is given in the Act.

The 1st Schedule to the Act contains two lists of diseases. Part I should be read in conjunction with Section 25 and includes the following diseases of cows: acute mastitis, actinomycosis of the udder, suppuration of the udder, any infection of the udder or teats which is likely to convey disease, any comatose condition, any septic condition of the uterus, anthrax, and foot-and-mouth disease. Part II should be read in conjunction with Section 37 and includes the following milk-borne diseases: enteric fever (including typhoid and paratyphoid fevers), dysentery, diphtheria, scarlet fever, acute inflammation of the throat, gastro-enteritis, and undulant fever.

The 3rd Schedule contains special provisions as to the sampling of milk which enable a milk purveyor whose milk has been sampled by a Local Authority to serve, within 60 hours, on that Authority a notice stating the name, etc., of the person from whom he received the milk and requesting the Authority to take steps to procure a sample of milk from a corresponding milking in course of delivery to himself. This procedure is of assistance to a milk purveyor who may wish to prove that the milk sold by him was in the same state as when he received it. The above provision does not apply when the original sample procured from the milk purveyor was from a mixture of milk obtained by him from more than one person.

Furthermore, when a sample of milk of cows in any dairy is procured in course of delivery, the dairyman may, within 60 hours, similarly request the Authority to procure a sample of milk from a corresponding milking of the cows, provided that the person procuring the sample shall take such steps at the dairy as may be necessary to satisfy him that it is a fair sample of the milk of the cows when properly and fully milked.

Finally, it should be pointed out that the addition of preservatives is automatically prohibited in all dairy products, as previously mentioned for milk when discussing Section 24, because these products are not listed in the Public

Health (Preservatives, etc. in Food) Regulations, 1925 to 1940, as being substances to which the addition of a permitted preservative is allowed.[1]

The Sale of Milk Regulations, 1939

These regulations were made by the Minister of Agriculture and Fisheries under the powers conferred on him by Section 23 of the Food and Drugs Act, 1938, and take the form of presumptive standards for milk and separated milk.

If a sample of milk (other than separated or condensed milk) contains less than 3 per cent of milk fat, it shall be presumed, until the contrary is proved, that the milk is not genuine, due to the abstraction of milk fat or the addition of water. Similarly, if a sample of milk contains less than 8·5 per cent of non-fatty-solids it shall be presumed, until the contrary is proved, that the milk is not genuine, due to the addition of water or the abstraction of non-fatty-solids.

There is no standard for the milk-fat content of separated milk, but the presumptive minimum standard for non-fatty-solids is 8·7 per cent.

It has already been mentioned that there is no official definition of milk given in the Act, it apparently being assumed that everyone knows what is expected when a demand for milk is made. It may, however, be inferred from decided cases on milk adulteration that as long as milk is sold in the same condition in which it was given by the cow, and that it has the general properties usually attributed to milk, any legal onus on the part of the vendor has been discharged. The above " standards " of quality have been fixed by the Ministry of Agriculture, but these are not standards as that term is ordinarily used, but *limits* below which it may be assumed, *until the contrary is proved*, that the milk is not genuine. Whether one agrees or does not agree that the law as it stands is equitable, it is beyond question that the Regulations were not framed in such a way as to prohibit the sale of milk having a composition inferior to the limits, and this view is amply confirmed by a series of cases that have been decided by the Divisional Court and the Court of Appeal, e.g. *Wolfenden v. McCulloch*, *Scott v. Jack*, *Hunt v. Richardson*, *Grigg v. Smith*. The case of *Hunt v. Richardson* has probably been more quoted in the Courts than any other case, and may be taken as an expression of the law on the subject as it is interpreted to-day. It should be remembered, however, that in more than one of these cases the Judges have stated, either expressly or by inference, that they gave their decisions with reluctance and, again, that they have not always been unanimous.

The case of *Hunt v. Richardson* (1916) was an appeal by way of case stated from a decision of the Justices of the Borough of Cambridge. It was deemed to be of such importance that a special Bench of five Judges sat to hear it. The decision was a majority one of three to two. The judgment was: " In this case a farmer contracted to sell new milk each morning to a purchaser who was a retail dealer. The milk was delivered in churns. Nothing was added to or abstracted from the milk. The herbage on which the cows producing the milk were fed was in a watery condition owing to heavy rain in previous months. As a consequence the milk produced was copious but inferior. To maintain the quantity of milk the cows were also fed on green maize. They were given in addition such an amount of cake each day as was usual under normal conditions. On analysis the milk in a churn delivered to the purchaser was found to contain less than 3 per cent of milk fat. The farmer was summoned before a Court of Summary Jurisdiction under S. 6 of the Sale of Food and Drugs Act, 1875, for selling to the prejudice of the purchaser milk which was not of the nature, substance and quality of the article demanded by the purchaser. The justices found that milk taken from a healthy herd should show not less than 3 per cent of milk fat, and that the deficiency was due to the manner in which the farmer fed his cows with the object of obtaining a very large supply of milk without regard to the quality thereof, and they convicted the farmer. On a case stated for the opinion of the King's Bench Division:

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[1] The Minister of Food under the Defence (General) Regulations, 1939, made an order called the Margarine (Addition of Borax) Order, 1940, which permitted under licence the addition of borates to margarine; a condition of the licence being that the amount added should not exceed 0·25 per cent expressed as boric acid.

> " Held by Darling, Lawrence and Avory JJ. (Bray and Scrutton JJ. dissenting), that the conviction should be quashed on the ground that there was no evidence of an offence under S. 6 of the Act.
>
> " Held by Bray and Scrutton JJ. that the case should be sent back to the justices.
>
> " By Bray J. that they might find whether the milk supplied was new morning milk of merchantable quality, that being presumably the quality demanded by the purchaser.
>
> " By Scrutton J. that they might find (1) what quality, if any, was demanded by the purchaser, and (2) whether the milk supplied was of that quality."

The stated case contained the sentence: " The quality of milk is affected by the quantity (of milk) and also by the state of health of the animal, the method of milking, and the manner of feeding, and the hours that elapse between successive milkings ". It is usually agreed now that the manner of feeding (provided that the cows have a reasonable diet) has far more effect on the quantity of milk than on its quality—thus Miscellaneous Publication No. 65 of the Ministry of Agriculture contains the sentences: " . . . it remains broadly true to say that where cows are suitably fed, milk cannot be altered appreciably in respect of the percentage of butter fat by the foods, and that as regards the solids-not-fat the effects are still more difficult to trace. . . . It is evident from this experiment that while starvation considerably affects the *quantity*, it only to a slight extent affects the *quality* in respect to the percentage of fat "; and it is further stated that research workers have been unable to find any evidence to support the view that feeding exerts any serious influence on the percentage of fat in the milk. This point is now so generally admitted that it would not be reiterated here, were it not for the use which is made—and, in the view of the revisers, improperly made—of the expression to the contrary which occurs in the statement of the case of *Hunt v. Richardson.* It is possibly useful to admit certain statements as true so that the whole attention may be given to another point which is considered to be of greater importance, but it should not be possible to insist on the absolute truth of any statement which has merely been allowed for the sake of removing one point of contention from the main argument. It is submitted that the case of *Hunt v. Richardson*, whilst being binding in certain respects, cannot be taken as proving something which is, in fact, not true: that is, that reasonable variations in feeding seriously affect the quality of milk.

The presumptive standards which appear in the Sale of Milk Regulations, 1939, were made after extended enquiry, because it was rightly thought that genuine milk from herds in nearly every case contains more than 3·0 per cent of fat and 8·5 per cent of solids-not-fat. The provision permitting proof to the contrary was inserted in order to allow for those few cases in which the composition of natural milk falls below the limits suggested. Where a milk naturally low in either fat or solids-not-fat is sold there is no offence, but the onus of proof lies on the defendant. It must not be thought that the limits of the Sale of Milk Regulations are in any sense an absolute standard for milk, or that milk which yields figures on analysis which are equal to, or slightly higher than these figures, is necessarily a good or genuine milk.

The figures given in the regulations merely enable one to describe any milk as adulterated, without further proof, if the composition is below the limit laid down, the onus of proof to the contrary being put on the defendant. It is quite possible, and not at all unlikely, that a milk may be adulterated in spite of having a composition equal to or better than that stated in the regulations. If proof of adulteration of any milk could be adduced, there is no doubt that conviction could follow, even for a milk the composition of which was superior to that specified in the regulations.

Reliance should not be placed solely upon the presumption of adulteration based on the Sale of Milk Regulations. When a sample of milk is found to be apparently adulterated, advantage should be taken of the powers of sampling given in Section 68 and the 3rd Schedule to the Act, and samples should be taken in course of delivery and then from the milk obtained after the cows have been milked in the presence of the sampling officer. The milk has now been traced back to its source, and the results obtained by analysis of these subsequent samples should be carefully compared with those of the original sample.

It occasionally happens that an individual cow, or even a herd of cows, gives milk which is somewhat below the limits fixed by the Sale of Milk Regulations. In such cases this fact will immediately be made known by the examination of the sample of milk which has been obtained from the cows in the presence of the inspector, i.e. an " appeal-to-cow " sample, and no proceedings are taken against the vendor. In the majority of cases, however, the milk obtained from the cows is reasonably good milk, with a composition considerably better than that of the regulations. The evidence of the appeal-to-cow sample is very valuable to the Court in making their decision in cases of prosecution.

The Condensed Milk Regulations, 1923 and 1927

These regulations deal with the composition and labelling of condensed milk. Condensed milk, as stated in Chapter 9, is prepared by heating cows' milk in a vacuum until between two-thirds and three-quarters of the water present has evaporated and the milk has become concentrated about two-and-a-half times. Both ordinary milk (described as " full cream " in the regulations and required to contain not less than 12·4 per cent of milk-solids)[1] and skimmed milk (required by the regulations to contain not less than 9·0 per cent of milk solids other than milk fat) are used in the preparation of condensed milk. In ertain cases cane sugar is added (the amount is usually about 40 per cent of the finished product), when the condensed milk is described as "sweetened". Although provision has been made in the regulations for the sale of condensed unsweetened skimmed milk this is not generally found in commerce, the three usual varieties being Full Cream Unsweetened, Full Cream Sweetened, and Skimmed Sweetened. The regulations require that the finished products as sold shall have compositions not inferior to those contained in the following table[1]—

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[1] Including not less than 3·6 per cent of milk fat. Where milk containing less solids than this is used, a proportionally larger amount will have to be taken in order to produce a condensed milk of the required concentration.

Table 14.1—Statutory requirements for condensed milk

Description of condensed milk	Percentage of milk fat	Percentage of all milk solids, including fat
(1) Full cream, unsweetened	9·0	31·0
(2) Full cream, sweetened	9·0	31·0
(3) Skimmed, unsweetened	—	20·0
(4) Skimmed, sweetened	—	26·0

The regulations also stipulate that where condensed milk is sold by retail, the container shall be labelled with a statement of the amount of standard reconstituted milk (i.e. milk containing not less than 12·4 per cent of milk solids, including not less than 3·6 per cent of milk fat) which can be prepared from the contents of the tin and, in the case of skimmed milk, a further declaration that this is "Unfit for babies".

The Dried Milk Regulations, 1923 and 1927

These regulations are similar in purport to the Condensed Milk Regulations. Dried milk is prepared by removing practically the whole of the water from cows' milk so that a dry white powder remains. Dried milk may be prepared from full-cream milk or from milk containing varying proportions of its fat. For the purposes of the regulations the terms "milk", "three-quarter-cream milk", "half-cream milk", and "quarter-cream milk" mean milk containing not less than the following percentages of milk fat and milk solids (including milk fat)—

Table 14.2—Standards for milks used in preparing dried milks

	Milk fat	*Milk solids*
Milk	3·6	12·4
Three-quarter-cream milk ..	2·7	11·6
Half-cream milk	1·8	10·8
Quarter-cream milk	0·9	9·9

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[1] The Minister of Food, under powers given to him under the Defence (General) Regulations, 1939, made an order called The Condensed Milk (Milk Content) Order, 1940, requiring full-cream unsweetened condensed milk to contain not less than 7·8 per cent by weight of milk fat and not less than 25·5 per cent by weight of all milk solids, including fat.

" Skimmed milk " means milk which contains not less than 9 per cent of milk solids other than milk fat. When milks of these descriptions are dried the products contain respectively the proportions of fat as set out in Table 14.3; the amount of water is usually less than 5 per cent, the remainder being made up of the non-fatty-solids of milk.

Table 14.3—Standards for dried milks

Type of dried milk	*Fat content per cent*
Full-cream milk	At least 26·0
Three-quarter-cream milk	,, 20·0
Half-cream milk	,, 14·0
Quarter-cream milk	,, 8·0

Dried milk containing less than 8·0 per cent of fat must be described as " Dried Skimmed Milk " and be labelled in a specified way which contains the statement " Unfit for babies ".

There are at present no statutory regulations in this country comparable to the above with regard to cheese or to cream.

The U.S.A. Federal and State standards for the composition of dairy products are given as ranges of minimum values—

Table 14.4

		Per cent
Milk	Fat	3 to 3·5
	Solids-not-fat	8 ,, 9
	Total solids	11 ,, 12
Skim-milk	Total solids	8 ,, 9·3
Cream	Fat	16 ,, 20
Butter	Fat	80 ,, 82·5
Condensed milk (whole sweetened)	Fat	7·7 ,, 8·5
	Milk solids	24 ,, 28
Evaporated milk (whole)	Fat	7·7 ,, 7·9
	Total solids	25·2 ,, 28
Ice cream	Milk fat	8 ,, 14
	Milk solids	18 ,, 20
Milk powder	Fat	26 ,, 27
Skim-milk powder	Fat	0 ,, 1·5
Cheddar cheese	Fat	50 (in dry matter)
Swiss cheese	Fat	43 to 45 (in dry matter)

The Milk (Special Designations) Order, 1936

This Order prescribed the following special designations for milk and stipulated the conditions under which they may be produced—

Tuberculin Tested milk.
Tuberculin Tested (Certified) milk.

Tuberculin Tested (Pasteurised) milk.
Accredited milk.
Pasteurised milk.

Tuberculin Tested milk

The special conditions under which tuberculin tested milk must be produced and sold are very briefly as follows—

(*a*) Each animal in the herd shall be submitted to a tuberculin test at an interval of not less than 2 and not more than 6 months after the preceding test, and the certificates of the result of the test must be sent to the licensing authority within 7 days.

(*b*) If an animal is certified as reacting to a tuberculin test it must be removed from the herd.

(*c*) All animals in the herd must be suitably marked and a register of the animals kept.

(*d*) Each animal shall be submitted to an examination by a veterinary surgeon at least every 6 months and the certificates of the examinations sent to the Licensing Authority within 7 days.

(*e*) If an animal is certified as suffering from any disease which will affect the milk injuriously, it must be segregated from the rest of the herd, and a register of such animals must be kept.

(*f*) The herd must be completely isolated from all other cattle.

(*g*) The milk must be consigned from the dairy in unventilated sealed containers, marked with the address of the dairy, the date of production, the word " morning " or " evening ", according to the time of milking, and the words " Tuberculin Tested milk ".

(*h*) Dealers in tuberculin tested milk must deliver it to consumers in the original sealed containers, in bottles, or in other suitable containers of not less than 2 gallons capacity. Each bottle must be tightly closed and sealed in a manner approved by the licensing authority, and it shall bear the address of the bottling establishment and the words " Tuberculin Tested milk "; if desired, certain additional wording, such as the date of production, etc., and the word " Certified ", if it has been bottled at the place of production, may be added.

(*i*) Tuberculin tested milk must not be heated or otherwise treated, except that where a licence for the use of the designation " Pasteurised " is held in relation to such milk it may be treated and labelled as " Tuberculin Tested Milk (Pasteurised) ".

Standards—Tuberculin tested milk shall not decolorise methylene blue within 4½ hours, if the sample is taken from 1st May to 31st October; or within 5½ hours if taken from 1st November to 30th April, when tested in the prescribed manner.

Accredited milk

The special conditions applying to the production and sale of " Accredited milk " are briefly as follows—

(*a*) Each milch cow in the herd shall be examined by a veterinary surgeon at least once every three months, and the certificates of the examinations sent to the licensing authority within 7 days.

(*b*) Every animal certified as suffering from a disease likely to affect the milk injuriously shall be segregated from the herd, and a register of such animals shall be kept.

(*c*) The herd shall not contain any animals which have been tested with tuberculin.

(*d*) All milch cows shall be suitably marked and a register kept.

(*e*) The milch cows in the herd shall be kept separate from all other cows in milk.

(*f*) The milk shall be consigned in an unventilated sealed container labelled as directed for tuberculin tested milk, except that the words " Accredited milk " shall be used.

(*g*) The conditions applying to the sealing and labelling of bottles by dealers are also similar to those indicated for tuberculin tested milk.

(*h*) " Accredited milk " must not be treated by heat, etc.

Standards—" Accredited milk " must satisfy the methylene blue test (see Tuberculin Tested milk).

The Milk (Special Designations) Order, 1949

The 1936 Order has been replaced by a new Order which, together with its regulations, now consolidates all the changes which have taken place since 1936. The most important material for the dairy chemist is contained in three sets of regulations. Two of these—The Milk (Special Designations) (Raw Milk) Regulations, 1949, and The Milk (Special Designations) (Pasteurised and Sterilised Milk) Regulations, 1949, are issued under the Order and now cover entirely the legal requirements for the four designated milks—Tuberculin Tested, Tuberculin Tested (Farm Bottled) Pasteurised and Sterilised. Together with the Milk and Dairies Regulations, 1949, these summarise the legal requirements which must be observed by all dairymen, and they have been aptly described as the " dairyman's bible ".

Broadly speaking, the Minister of Agriculture and Fisheries now controls the production of milk and the Minister of Health acting jointly with the Minister of Food controls the sale as far as technical and public health aspects are concerned. Some important changes are made in the new regulations. The use of " Accredited " as a designation will cease on the 1st of October 1954, and the designation " Certified " is now replaced by " Farm Bottled ". The coliform test, which could previously be applied to raw designated milks, is now omitted, and the only statutory test for these milks is the methylene blue test, the method of carrying out the test being unchanged.

As from October 1952, Tuberculin Tested milk may only be produced from herds which are on the Ministry of Agriculture's Register of Attested Herds.

The regulations for heat-treated milk apply to milk which may be pasteurised by three different methods and to sterilised milk which now for the first time becomes a designated milk. Milk may be pasteurised—

(i) by being held for 30 minutes at a temperature of from 145° to 150° F (62·8°–65·5° C);
(ii) by being held at a temperature of at least 161° F (71·6° C) for 15 seconds; and
(iii) by any other method which is approved by the licensing authorities.

With all three methods the milk must be cooled to a temperature of 50° F (10° C)—previously 55° F (12·8° C)—as soon as possible. A flow-diversion device must be fitted to all pasteurising equipments in which the milk is heated to a temperature of 150° F (65·5° C) or higher.

Pasteurised milk is subject to two statutory tests—the methylene blue (for keeping quality) and the phosphatase (for efficiency of pasteurisation) tests, the techniques for which are practically identical with the previous ones.

The regulations lay down provisions for the setting up of tribunals to which a dairyman may appeal if he wishes.

A detailed description of techniques for making these statutory tests is given in Chapter 26. See also Davis's *Milk testing*, the article by Barton on " Legal aspects " in Davis's *Dictionary of dairying*, and Barton's *Dairy law*.

PART II

ANALYTICAL METHODS

NOTE

No analysis is worth anything unless the sampling is adequate. A most useful list of sampling procedures has been issued by the British Standards Institution (" Sampling of dairy products ", B.S. No. 809—1949). See also the article on " Sampling " in Davis's *Dictionary of dairying* and the chapter on " Sampling " in Davis's *Milk testing*. B.S. No. 1416 and 1417 (1948) deal with the sampling and analysis of acid and rennet caseins.

CHAPTER 15

SPECIFIC GRAVITY, TOTAL SOLIDS, ASH, FREEZING POINT AND ACIDITY OF MILK

(1) THE DETERMINATION OF SPECIFIC GRAVITY

There are two methods of determining specific gravity: we may determine the weight of a known volume, or the volume of a known weight. The former method is used as follows. (1) A vessel of known volume is filled with the liquid and its weight taken, or (2) a weighed plummet of known volume is immersed completely in the liquid, and the loss of weight due to the displacement of an equal volume of liquid noted. The latter method is applied (3) by immersing a float of known weight, and noting the volume immersed; the volume immersed will be equal to a volume of the liquid of weight equal to that of the float.

Determinations of specific gravity by method (1) are made with specific gravity bottles and Sprengel tubes, by method (2) with a Westphal balance, and by method (3) with hydrometers, of which lactometers are merely special forms of limited range suitable for milk.

The Sprengel tube

For exact determinations of the specific gravity of milk, a Sprengel tube presents many advantages. It is a U-shaped tube with narrow capillary ends bent outwards at right angles, one being rather smaller than the other; the wider of the two has a fine line etched round it, to which the liquid in the tube may be adjusted, the U and the other capillary being completely filled.

The weight of the dry and empty tube is first ascertained, the tube is then filled with pure distilled water, and immersed in water at 15·5° C (60° F); when it is seen that no further expansion or contraction takes place, the water should be adjusted to the line on the wider capillary by the cautious application of a piece of filter-paper to the end of the narrow capillary; the tube is then wiped dry and weighed. The difference of the two weights gives the weight of the water contained in it. The tube is then filled with milk and immersed in water at 15·5° C, and the milk similarly adjusted to the line; the weight of milk divided by the weight of water gives the specific gravity of the milk at 15·5°/15·5°.

A specific gravity bottle is used in a similar manner; the liquid, after inserting the stopper and immersing the bottle in water at 15·5° C, is adjusted to the line by drawing out the excess with a very fine tube.

A Sprengel tube of 10 to 20 ml capacity is the most suitable size; it is a disadvantage to use a larger one, as the time taken for the milk to assume the temperature of the surrounding water is so much increased that there is danger of a portion of the cream separating.

The advantages of a Sprengel tube over a specific gravity bottle are—

(1) Greater surface for a given volume; and therefore the temperature is adjusted more quickly.

(2) There is no stopper to be fitted; consequently, no error can be due to difference of position owing to inaccuracy of fit.

It is not proposed to describe the Westphal balance in detail here, as it is not as accurate as the Sprengel tube, nor is it as convenient as the lactometer.

The lactometer

For practical convenience in determining specific gravities of milk, the lactometer is undoubtedly the most useful of the instruments available, and it is the one almost universally used in one form or other in dairy work.

The faults of lactometers are—

(1) They do not indicate true specific gravities, but the inverse of this, viz. specific volumes; consequently the scale is not divided into equal parts. The divergence from equality is, however, so small in a lactometer, which has only a limited range, as to render it practically admissible to treat the smaller divisions as equal.

(2) The exact point at which the level of the liquid cuts the stem of the lactometer cannot be ascertained, as, owing to surface energy, the liquid is attracted to a higher level round the stem of the lactometer than the surface of the liquid; moreover, the height to which the liquid is attracted varies with the nature of the liquid. As, however, milk has always the same composition within narrow limits, there is no practical difference in the height to which it is attracted round the stem; the eye soon becomes trained in making the proper allowance for this.

(3) Lactometers are only correct at the temperature at which they are graduated; at other temperatures their volume varies; but no inconvenience on this account is found in practice, as this is allowed for in the tables given for correcting the specific gravity to a temperature of 15·5° C.

Soxhlet's lactometer consists of a stem carrying a scale, on which the specific gravity is read, a cylindrical body, and a bulb at the lower end containing shot. A very convenient form carries a scale divided in tenths of a degree from 27° (sp. gr. 1·027) to 35° (sp. gr. 1·035). For greater variations in specific gravity it is possible to obtain a series of lactometers covering an extended scale from, say, 21° to 28°, 28° to 35°, and 35° to 41°.

Vieth's lactometer and Quevenne's lactometer are other modifications in which the shape of the body and the range of the scales are different.

The thermo-lactometer and Gerber's lactometer combine two scales, one of which reads the specific gravity and the other the temperature on the enclosed thermometer. These lactometers are usually neither as small nor as delicate as the ordinary lactometer; they are therefore more suitable for use in the dairy than in the laboratory, where samples are often limited in bulk.

Use of the lactometer

It is advisable to wait until several hours after milking before attempting to obtain a specific gravity figure, because of the varying results, due to what is known as Recknagel's phenomenon, which are obtained with freshly-drawn milk (see p. 112).

Each milk sample should be mixed gently just before taking the specific gravity, in order to mix in the cream, but care must be taken to avoid the formation of air bubbles. The following directions are due to Vieth.

In order to determine the specific gravity, the milk is poured into a vessel at least ¼ inch greater in diameter than the widest part of the lactometer, and deep enough to allow the instrument to float. A cylindrical glass jar, with foot, is the most suitable vessel for the purpose if Soxhlet's lactometer or the thermo-lactometer be used; Vieth's lactometer may be used in a can or tin cup. The lactometer is gradually lowered into the milk to the 25th degree, care being taken that the instrument is entirely wetted by the milk and that no air adheres to it. When released, the lactometer will move up and down, and after a little while becomes stationary. That degree of the scale which coincides with the surface of the milk is then noted. It will be observed that where the milk touches the vessel and the stem of the lactometer, the surface is not level, but, in consequence of the adhesion of the milk to the glass, forms a curve. There is no difficulty, however, in ascertaining the extension of the curve sufficiently near, and this has to be allowed for in reading off the specific gravity.

Lactometers indicate the exact specific gravity at a temperature of 60° F (15·5° C). It is therefore necessary, as soon as the position of the lactometer has been noted, to remove the instrument from the milk, immerse a thermometer, and ascertain the temperature.

If the temperature is found to be 60° F, the observed specific gravity is correct, but should the temperature of the milk be higher or lower than 60° F, the specific gravity must be corrected by the aid of Richmond's Milk Scale (p. 160) or Table 5.4 on p. 111; the latter is used as follows—

Find the temperature of the milk in the vertical column, and the observed specific gravity in the first or last horizontal line; under the latter, and in the same line with the temperature, is given the correction to be added if the milk is above 60° F and subtracted if below. For example: supposing the temperature to be 51° F and the specific gravity 34°, the correction to be subtracted is 1·1, giving the specific gravity corrected to 60° F as 32·9° = 1·0329; or if the temperature is 66° F and the specific gravity 29°, the correction to be added is 0·8 and the specific gravity is 29·8° = 1·0298. An approximation can be made to the gravity at 60° F by adding or subtracting 0·1 for every degree over or under 60° F.

Never take the specific gravity of a milk without also noting the temperature and correcting to 60° *F.*

Instead of reading from the bottom of the curve and making a mental allowance, the lactometer may be read from the top of the curve and a definite figure (ascertained by a few carefully conducted experiments) added on.

As soon as the specific gravity and temperature have been taken, the corrected specific gravity from the table should be entered in the book provided for the purpose of recording the results. It is not necessary to enter the specific gravity in full, but only the three significant figures; thus a specific gravity of 1·0325 may be entered simply as 325 or 32·5.

Though the determination of specific gravity has been described first, it is found that when total solids are to be estimated, it is convenient in practice to commence work with their estimation, as this is an operation which may then proceed alone. Only in cases where the sample is so small that the lactometer will not float conveniently, if the quantity for total-solid estimation has been removed, is it necessary to take the specific gravity first.

Ystgaard *et al.* (1951a) have compared various methods of measuring the amount of added water in milk, using lactometer methods with different formulae and the freezing point method. All lactometer methods yielded low results, the variations from the actual percentage of added water increasing non-linearly with the increase in added water. Ystgaard *et al.* (1951b) have also compared the accuracy of various lactometer methods for the determination of total solids in normal and adulterated milk.

Hutchinson (1942b) has extended the Richmond table to cover the range 40° to 90° F and also evolved an equation to cover this range. Humphriss (1948) has described a form of lactometer jar convenient for rapid testing. A densimeter cylinder has also been described by Martin (1948).

Calibration of lactometer

This can be conveniently done by taking the specific gravity at 15·5° C of several samples of milk, both by lactometer and by an accurately calibrated Sprengel tube. The milks chosen should have a fairly wide range of specific gravities, and any lactometer which shows differences between the two methods of more than 0·2° should be rejected, unless the error is a constant one over the whole range of the scale, when a correction may be applied.

Alternatively, suitable salt solutions may be prepared; for example, 3·863 per cent pure sodium chloride solution has a gravity of 1·028 at 15·0° C, and 4·415 per cent salt solution has a gravity of 1·032 at the same temperature. The specific gravities of these are checked by means of a Sprengel tube, and then the lactometer readings are taken in exactly the same way as with milk.

Determination of density

This can be carried out in a similar manner to the determination of specific gravity: for example, by the use of a Sprengel tube, which will, however, be calibrated in terms of millilitres at a definite temperature, instead of (as when specific gravity is determined) grams of water at a definite temperature.

In practice, however, the determination is most easily carried out by the use of a density hydrometer, which is simply a hydrometer or lactometer calibrated to give results in terms of density under specified conditions; the method of carrying out the determinations, subject to minor variations, is the same as that employed in determining specific gravity. The determination of density has, however, the great advantage of giving results which are in terms of absolute units at one specified temperature, and it is not subject to the confusion which has arisen through the use in the past of numerous conventions for indicating specific gravity under a variety of conditions; a density hydrometer also has the additional advantage of requiring the application of only relatively small corrections when it is used at temperatures other than that for which it was calibrated.

Density hydrometers particularly suitable for the determination of the density of milk are described in B.S. No. 734—1937,[1] and reference should be made to this specification for details of their construction and use. These density hydrometers are adjusted to indicate density in grams per millilitre at 20° C in a liquid having a surface tension of 46 dynes per cm (the average surface tension of milk at 20° C). The specification includes tables for use in conjunction with the hydrometers, including a table for the determination of total solids and solids-not-fat from the fat percentage and density figure. This last table is based upon Richmond's formula for the determination of percentage total solids from the fat percentage and specific gravity, which has simply been modified in terms of density, expressed in grams per millilitre at 20° C, instead of the original form based upon specific gravity S 60° F/60° F at 60° F.

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[1] Published by the British Standards Institution, 28 Victoria Street, London S.W.1.

The revised formula is

$$\text{T.S.} = 0{\cdot}25\, D + 1{\cdot}21\, F + 0{\cdot}66$$

In order to avoid any inconsistencies due to incomplete Recknagel contraction (page 112), it is recommended in the new technique that all samples shall be held at 40° C for five minutes and cooled to 20° C before taking the density. By so doing the Recknagel contraction is eliminated.

Boden and Campbell (1942) have pointed out that Richmond derived his formula from results obtained on milks in which the Recknagel change was complete, and that the formula used in the new method should include a factor to compensate for the loss of this effect. Figures published by these two workers demonstrate that the new technique gives solids-not-fat results which are, on the average, 0·2 per cent below those obtained by the original specific gravity method or by the weighed total-solids method. The use of the new technique was found to place an abnormally large proportion of samples below the presumptive legal standard of 8·5 per cent solids-not-fat. See also Walker's (1945) results on homogenised and sterilised milks.

An amendment to B.S. No. 734 (Amendment No. 1, February 1946) has been issued which states that

> " . . . the results obtained will be slightly lower than those obtained hitherto by an amount depending on the extent of the Recknagel contraction of the unwarmed samples. The adoption of the pre-warming procedure is recommended as it has been found by practical tests that it ensures reproducible results and when used in conjunction with Table C [of the Specification] yields results for total solids which are in satisfactory agreement with those obtained by direct gravimetric determinations."

Richmond's formula was calculated on the basis of " best fit " on the results of tests of some 240 samples taken at different times of the year. He did not state whether the Recknagel effect was definitely completed with these samples. Bowden and Campbell (1942) found that refrigerated samples gave a correct result by lactometer using the gravimetric determination as a basis, and hence they believe that Richmond's formula gives correct results when the Recknagel effect is completed.

Rowland (1951b) has found that the Richmond formula, used for samples in which the Recknagel effect has been completed, gives results which are about 0·2 per cent solids-not-fat (equivalent to 0·8 lactometer degrees) higher than those obtained by the B.S.I. method. Rowland finds that the gravimetric method gives a solids-not-fat value about 0·15 per cent solids-not-fat lower than the Richmond method with the Recknagel effect completed, and about 0·04 per cent solids-not-fat higher than the B.S.I. method with no Recknagel effect. The B.S.I. formula is really a corrected Richmond formula to make the necessary adjustments for the change of temperature from 60° F to 20° C. It is not concerned with the Recknagel effect as such. The warming of the sample which is a feature of the B.S.I. method may therefore reduce the solids-not-fat result by an amount up to 0·2 per cent. It may be assumed that the Recknagel change probably occurs to only a negligible extent in summer, even when the samples are 18 hours old, but is probably almost completed in this time in cold winter weather. Hence it is reasonable to assume that the ordinary Richmond method without any special warming up or refrigeration of the samples will give results in winter which may be up to 0·2 per cent higher than those obtained in summer.

Testing of density hydrometers

The fact that a hydrometer is marked with the number of the above specification should not be taken as a guarantee that it necessarily conforms with the limits of the specification, and it is recommended that advantage should be taken of the facilities of the National Physical Laboratory, with a view either to the verification of the fact that the hydrometer is accurate within the prescribed limits, or for a certificate indicating in addition the corrections which may be applied to the readings.

Finally, it should be noted that in milk analysis the determination of specific gravity or density is normally carried out with a view to the correlation of this figure with the percentage of fat arrived at by a volumetric process, thereby obtaining, with the use of a formula or tables, a quick determination of the percentage of total solids and solids-not-fat in the sample. For this purpose these determinations are invaluable, and the results obtained are sufficiently accurate to act as sorting tests. In cases, however, where from the above tests the milk appears to be either adulterated or of doubtful quality, direct gravimetric determinations of total solids should invariably be carried out.

(2) DETERMINATION OF THE TOTAL SOLIDS OF MILK

The total solids of milk are determined by evaporating the water and weighing the residue.

S.P.A. method (Soc. Publ. Anal. 1945a)

Weigh about 5 g of the thoroughly mixed sample of fresh milk (note (*a*)) in a dry (*b*), round flat-bottomed metal dish (*c*), about 7 to 8 cm in diameter and 2 cm in depth, and provided with a readily removable but closely fitting lid which is weighed with the dish. Place the dish, uncovered, on a rapidly boiling water-bath (*d*) for 30 minutes. Wipe the bottom of the dish and transfer the dish and lid to a well-ventilated oven at 98° to 100° C (*e*), as recorded by a thermometer in the air immediately above the dish. After three hours in the oven (*f*), cover the dish before removal from the oven and transfer it to a desiccator with an effective desiccant, using a separate desiccator for each dish. Cool for 30 minutes (*f*), and weigh dish with lid. Return dish and lid to the oven and heat for one hour, with the dish uncovered. Remove to desiccator, cool and weigh as before. Repeat the re-heating, etc., if necessary, until the loss of weight between successive weighings does not exceed 0·5 mg.

Notes

(*a*) The foregoing method applies to milk in a *fresh* condition, i.e., milk which has not undergone appreciable change. When the acidity—phenolphthalein being used as indicator—exceeds 0·20 per cent expressed as lactic acid, a slight loss of acid may occur on drying, and the quantity of strontium hydroxide solution necessary to neutralise the sample should be added before heating, a deduction being made for the added strontia (1 ml of 0·1 N $Sr(OH)_2 \equiv 0{\cdot}00428$ g).

(*b*) The empty dishes and lids should be dried immediately before use by heating in the oven for not less than 30 minutes and cooling in the desiccator for 30 minutes.

(*c*) Dishes made of aluminium, platinum, nickel, or stainless steel are suitable; blank determinations with water should be made to establish that the dishes are not altered in weight by the treatment.

(*d*) Dishes should not come into contact with the metal of the water-bath, and they should be in a horizontal position, so that the milk solids will form a uniform film.

(*e*) In electric ovens the temperature may be low near the bottom and front, and much over 100° C near the walls, especially at back corners; also, by conduction of heat through the metal of the dish and shelf, the milk solids adhering to the dish may be overheated. Dishes should not be placed near the walls of the oven, and should be insulated from the shelf, e.g. by a silica or glass triangle. The shelf used should be near the middle of the oven.

(*f*) The oven and desiccator should not be opened during the periods of heating and cooling respectively.

Golding (1934) used aluminium dishes or bottle caps about 44 mm in diameter and weighing about 1·2 g. In these he weighed accurately about 1 g of milk. After drying on the steam bath for 15 minutes, the dishes were placed in an air-oven maintained at the temperature of boiling water for 2 to 3 hours, weighed, replaced in the oven for a further hour, and weighed again. With the use of an air-damped balance it is possible to weigh 30 to 40 of these dishes containing total solids in 30 minutes.

Richmond (1920) found that by taking small quantities of milk—about 1 g—and spreading over a large surface during evaporation, a nearly white residue was obtained, and that constancy of weight could also be obtained.

Aschaffenburg (1938) has described a rapid method for determining the total-solids of milk, using a special electrical drying oven known as the " Carter-Simon rapid moisture-tester ", which was originally devised for the determination of moisture in cereals. For the test the oven is adjusted to 148° C. By using approximately 1 g of the sample and following the technique described in the original paper, complete duplicate determinations can be carried out in less than half-an-hour. A series of results are given which show very good agreement on comparison with results obtained by the method of Golding, *above*.

Previous attention has already been directed to the importance of using drying ovens which can be maintained at a constant temperature. In order to do this, Tate (1934) used a fan, mounted in a vertical position on the inside of the door of an electric oven of the normal type; this ensures even ventilation and therefore even temperature throughout the interior of the oven.

Babcock's method. Babcock has used asbestos as a medium over which to spread the milk. The method as adopted by the Association of Official Agricultural Analysts (of America) is described elsewhere (A.O.A.C. 1950). Richmond found Babcock's method most satisfactory and convenient to operate as follows—

Place about 3 g of fine asbestos fibres in a small platinum basin, and ignite strongly (preferably in a muffle). The asbestos should be soaked in hydrochloric acid and thoroughly washed before use; when ignited and shaken with water containing a few drops of phenolphthalein, no red colour should be produced. After weighing, add about 5 g of milk and weigh again as quickly as possible to the nearest milligram. Place the basin for an hour or two on a water-bath, and dry in a water-oven till constant in weight.

The residue thus obtained shows no signs of browning, and a constant weight, which shows no appreciable change on prolonged drying, can be obtained. The " total solids " by this method are somewhat hygroscopic, and care must be exercised in weighing.

Hawley (1933c) used flat-bottomed weighing-bottles, half filled with acid-washed, blue asbestos fibre; these are dried overnight over sulphuric acid in an exsiccator exhausted to about 2 mm vacuum. Approximately 5 g of milk are then accurately weighed into each weighing bottle. The stoppers are placed edgeways on the tubes and they are dried overnight in a 6-inch Hempel exsiccator, exhausted to full vacuum and containing 20 ml of fresh concentrated sulphuric acid for each milk tube. The following morning the exsiccator is opened and the lids of the tubes immediately placed in position, after which the tubes are weighed. The method overcomes the difficulty of attempting to weigh hygroscopic milk solids in open dishes under conditions of high humidity.

Ystgaard *et al.* (1951c) have compared three methods for the determination of total solids in milk—the A.O.A.C., the Mojonnier and the Brabender. They suggest that for the last method a 10 g sample, a drying temperature of 110° C, and a three-hours drying time give the best results. Total-solids values were significantly lower than those obtained by the A.O.A.C. method, the mean difference being 0·19 per cent.

The variations between replicate determinations were smallest with the Brabender and greatest with the A.O.A.C. method. These workers obtained no significant difference between mean determinations, using the Mojonnier and A.O.A.C. procedures.

(3) DETERMINATION OF THE ASH OF MILK

It is preferable to estimate the ash on at least 10 g of milk. Where the total-solids have been determined on a weighed 5·0 ml of milk, it is convenient to add to the total solids a further 5·0 ml of the sample, pipetted in exactly the same way as the first 5·0 ml; evaporate this to dryness on the water-bath for about an hour; then carefully char the residue over a burner, and finally ignite gently to a white ash in a muffle. Under these conditions, the weight of the ash multiplied by 100 over twice the weight of the sample taken for the determination of the total solids will give the percentage of ash. The temperature of the muffle must not be allowed to rise above a barely perceptible red heat, or distinct volatilisation of alkaline chlorides will occur.

If difficulty is experienced in obtaining a white ash, the following procedure will be found helpful. The dish is allowed to cool; the ash is then moistened with water and the dish placed upon a boiling water-bath until the contents are perfectly dry; on repeating the ignition in a carefully regulated muffle furnace, a perfectly white ash is almost invariably obtained.

A more exact determination is obtained by evaporating a larger quantity of milk than is usually taken for the total solids estimation, e.g. 25 to 50 g, and igniting gently until thoroughly charred; the mass is extracted with hot water and filtered through an ashless filter-paper, the insoluble portion and the filter being (after washing) ignited at a red heat until white: this will give the insoluble ash. The filtrate is evaporated and ignited cautiously at a low temperature; this gives the soluble ash. The sum of the insoluble and soluble ash gives the total ash; the results obtained in this way are usually slightly higher (about 0·02 per cent) than the ash obtained by ignition of the total solid residue. The above procedure is advisable if it is desired to examine the ash further, but if the total ash figure only is required it will be sufficient if, after the insoluble ash has been ignited to a white ash, the filtrate is added to and evaporated and ignited in the same dish, thus confining the operation to one weight determination.

It should be noted in passing that in the analysis of sour milk, where total solids have been determined by first neutralising with 0·1 N caustic soda or strontia, the total solids cannot be used for the determination of the ash, but a separate weighed portion of the sample should be evaporated for this purpose.

The following scheme enables the chief constituents of the ash to be determined—

The alkalinity may be determined by adding 1 drop of methyl orange indicator to the solution of the soluble ash obtained above and titrating with 0·1 N sulphuric acid, the result being expressed in terms of either Na_2O or Na_2CO_3.

Chlorides may be determined in the solution after the determination of alkalinity by adding potassium chromate indicator and titrating with 0·1 N $AgNO_3$, the result being expressed in terms of either sodium chloride or chlorine.

The insoluble ash is dissolved in a slight excess of dilute hydrochloric acid, and the solution (nearly neutralised with ammonia if necessary) heated to boiling; a cold saturated solution of ammonium oxalate is dropped in slowly till the addition of a further drop gives no further precipitate. After standing at least two hours, the precipitate is filtered off, washed, and ignited at a low temperature to convert the oxalate into carbonate; it is best to moisten the ignited precipitate with ammonium carbonate solution and to re-ignite at a very low temperature. The precipitate, after weighing, is dissolved in dilute hydrochloric acid, keeping the bulk small; ammonia is added to alkaline reaction, and the small precipitate of calcium phosphate collected, ignited and weighed. Its weight is subtracted from the previous weight, and the difference gives the weight of the calcium carbonate, which multiplied by 0·4 gives the calcium, or by 0·56 the lime, contained in it; the weight of the calcium phosphate multiplied by 0·3871 gives the calcium, or by 0·5419 the lime contained in it. The total calcium or lime is the sum of the two.

The filtrate is made strongly ammoniacal by the addition of 0·880 ammonia and allowed to stand 24 hours. The precipitated magnesium-ammonium phosphate is filtered off, washed with dilute ammonia, ignited, and the magnesium pyrophosphate weighed. Its weight multiplied by 0·21622 will give the magnesium, and by 0·36036 the magnesia contained in it.

To the filtrate from this, magnesia mixture is added. The precipitate of magnesium-ammonium phosphate is filtered off after 24 hours and treated as above.

From the total weight of the two quantities of magnesium pyrophosphate, the phosphoric anhydride is calculated by multiplying by 0·63964; to this is added the phosphoric anhydride in the calcium phosphate, calculated by multiplying the weight by 0·4581.

The above method has proved satisfactory in Richmond's hands, though it takes no account of the trace of iron present, which is precipitated with the calcium phosphate or with the magnesium-ammonium phosphate. If desired, this may be estimated by dissolving up the precipitate of calcium phosphate and the first magnesium-ammonium phosphate precipitate in dilute hydrochloric acid, and determining the iron colorimetrically as thiocyanate.

To estimate alkalis, another portion of milk is ignited as before, and the total ash dissolved in dilute hydrochloric acid and boiled; a few drops of barium chloride solution are added, containing not more than 0·1 g of barium to 100 g of milk, and the boiling is continued for some minutes. After some hours the precipitate of barium sulphate is filtered off, ignited and weighed; its weight multiplied by 0·34335 will give the sulphuric anhydride in the milk. If an excess of barium chloride has been added, a little phosphoric acid, or ammonium phosphate, may be dropped into the filtrate, though it is not necessary if the quantity of barium chloride given above has been employed. A quantity of ferric chloride solution sufficient to colour the solution brown is added, and the filtrate made alkaline with ammonia. The precipitate is washed well, and the filtrate evaporated and ignited very cautiously; the weight of the evaporated and ignited filtrate and washings will give the alkali chlorides. The residue is dissolved in water, and the solution should be quite clear; if it is not so, a little ammonium carbonate is added, the liquid evaporated to dryness, and the residue ignited cautiously; the residue is again taken up with water, the solution filtered and evaporated, and the residue ignited cautiously and weighed.

The chlorine in this may be titrated by standard silver nitrate, using potassium chromate as indicator. The potassium and sodium are calculated by the following formula—

Let W = weight of alkali chlorides
and C = weight of chlorine therein

Then the weight of sodium $= 2{\cdot}997C - 1{\cdot}4254W$
and ,, ,, potassium $= 2{\cdot}4254W - 3{\cdot}997C$

The potassium may be estimated directly by evaporating the solution of alkali chlorides with an excess of platinum tetrachloride solution almost to ryness; the pasty residue is treated with 80 per cent alcohol containing about per cent of ether, and washed repeatedly with this; the alcohol is passed hrough a weighed filter or preferably a Gooch crucible, and the precipitate is inally transferred to this and washed with ether. It is then dried at 100° C and weighed; the weight multiplied by 0·3056 will give the potassium chloride; this, subtracted from the weight of the alkali chlorides, will give the sodium chloride.

The potassium chloride multiplied by 0·5244 will give potassium, and by 0·6314 potassium oxide (K_2O). The sodium chloride multiplied by 0·3932 will give sodium, and by 0·5299 sodium oxide (Na_2O).

The above scheme of analysis has been worked out so as to use as little milk as possible, as the amount available is sometimes limited. Many obvious modifications are available and will readily suggest themselves to analysts; thus the chlorine may be estimated gravimetrically, or the perchlorate method used for potassium, and, if the amount of milk be sufficient, the phosphoric acid may be separated from another portion by the molybdic acid method.

The above method of determining the chlorides suffers from the disadvantage that the milk must first be ignited, and volatilisation of chlorides is therefore liable to occur. Davies (1932b) has described the following method which has the additional advantage of rapidity. Ten grams of milk are weighed into a 250 ml flask. Ten ml of 0·05 N $AgNO_3$, 2 ml of 6 per cent $KMnO_4$ solution and 10 ml of pure concentrated nitric acid are then added and the contents of the flask are boiled until the liquid is clear and reddish-brown fumes are copiously evolved. This usually takes from three to four minutes. A small quantity of urea is added to the hot solution, which is then diluted to 100 ml. Six ml of acetone and 10 ml of a saturated solution of iron alum in 10 per cent nitric acid are added and the excess of silver nitrate is titrated with 0·05 N potassium thiocyanate. The silver nitrate and the potassium thiocyanate solutions are standardised by means of standard potassium chloride solution, and at the same time a blank determination for the reagents is also carried out. The reagents should, however, be absolutely free from chlorides.

(4) DETERMINATION OF THE FREEZING POINT OF MILK

The most convenient apparatus for the determination of the freezing point of milk is the Hortvet cryoscope, and certain details of the technique adopted in using this instrument have already been discussed in the section on the freezing point of milk. The method of carrying out this test is described in detail below.

Before determinations are made on milk it is necessary to check the Hortvet thermometer by determining the freezing point of sugar solutions. The A.O.A.C. found, by the use of thermometers calibrated at the American Bureau of Standards, that the freezing point of a solution of 7 g of sucrose in 100 ml of water made up at 20° C was −0·422° C, whilst that for a similar solution containing 10 g in 100 ml was −0·621° C. The A.O.A.C. then directs that use be made of the corrections found at these two points on the actual thermometer in use, to calibrate that portion of the stem lying between them. It is apparent that any irregularities in the stem of the thermometer between these two points would lead to incorrect results, and Elsdon and Stubbs (1931) therefore suggested that for the most accurate work other sugar solutions should be tested, containing 7·5, 8·0, 8·5, 9·0 and 9·5 g of sucrose, respectively, in 100 ml of water. The freezing points of these intermediate solutions are given in the last column of Table 5.14, on p. 130.

Elsdon and Walker (1942) found that sugar solutions of 9 % or greater concentration have a distinct tendency to freeze before they have supercooled to the desired amount. It was suggested that filtration of these concentrated solutions might remove traces of suspended matter and thereby enable supercooling to take place, but consistent results were not obtained by adopting this procedure. For routine checking of the thermometer, they determined the freezing point of 7 g per 100 ml and 8·5 g per 100 ml solutions, and considered the latter solution to be of particular importance as its freezing point approximates to that of milk of borderline quality.

Although it is not necessary to check the freezing points of sugar solutions every day, it is essential that, on every occasion when the instrument is used to determine the freezing point of milk, the zero correction of the thermometer should also be determined by means of recently-boiled distilled water. Should this figure be seriously different from the zero correction obtained on the last occasion when the freezing points of the sugar solutions were found, the latter should be again determined and the new correction applied.

The standardisation of the Hortvet thermometer by means of sucrose solutions has been criticised by Aschaffenburg and Hall (1949) who found that unreliable results might be obtained with concentrations greater than 8 per cent. They recommend standardisation on the International Temperature Scale at the National Physical Laboratory or some similar testing institution.

The acidity of every sample should be determined at the time of testing, and a correction applied to the result obtained on any milk with an acidity which exceeds 0·18 per cent calculated as lactic acid. The original freezing point of the sample may then be calculated from the following formula (Scotland 1945)—

$$\text{original F.P. °C} = \text{observed F.P. °C} - 0{\cdot}34\,(\text{per cent lactic acid w/w} - 0{\cdot}18).$$

It is not advisable to attempt an estimate of the original freezing point of a milk if the acidity exceeds 0·30 per cent lactic acid, but to obtain it by the method of Evans (1936) or that of Macdonald (1945).

It is often necessary to send samples for freezing-point tests through the post or by rail, and on such occasions it is as well to add some preservative. Lockwood (1932) has recommended the use of 1 ml of a solution containing 18 ml formalin (38 per cent H.CHO) per litre in 100 ml milk. Another suitable isotonic solution consists of 1 per cent mercuric chloride and 0·85 per cent sodium chloride.

Macdonald (1943) has described the following method of obtaining the original freezing point of milk containing formalin by estimating the proportion of formaldehyde and applying the appropriate correction to the observed freezing point. Fifty millilitres of milk are pipetted into a 100 ml graduated flask, followed by 30 ml water, 4 ml 2N acetic acid, and 2 ml 15 per cent phosphotungstic acid solution, the contents of the flask being mixed by gentle swirling after each addition. The mixture is made up to volume, mixed, left for 10 minutes, and filtered, the first few millilitres of filtrate being rejected.

Twenty-five ml of filtrate are pipetted into a 250 ml glass-stoppered flask, 5 ml N sodium hydroxide and 20 ml 0·2 N sodium sulphite added, and the mixture allowed to stand for 15 minutes. After acidification with 2 ml of 2N acetic acid, 3 drops of 10 per cent potassium iodide solution and 1 ml of 1 per cent starch solution are added as indicators and the excess sulphite titrated with 0·1 N chloramine–T. The sulphite solution is standardised against the chloramine–T in the presence of a 25 ml aliquot of an unpreserved milk filtrate, the same procedure being used throughout. From the difference in titre of this blank and the test solution, the formaldehyde is calculated as follows—

$$1 \text{ ml } 0{\cdot}1 \text{ N chloramine–T} \equiv 1{\cdot}5 \text{ mg. H.CHO}$$

The end-point of the titration, although fading off after 30 seconds, is quite definite.

A correction for the volume of the precipitate can be calculated from the fat and solids-not-fat of the milk by the following formula—

$$\text{Volume of precipitate for 50 ml milk} = 0{\cdot}56 \times \text{fat per cent} + 0{\cdot}15 \times \text{S.N.F. per cent.}$$

For every 100 p.p.m. of formaldehyde present 0·009° C is subtracted from the observed freezing-point depression.

The Hortvet apparatus and technique

The apparatus is thus described by the A.O.A.C. (*Methods of Analysis*, 7th edn., p. 235). A cylindrical-shaped Dewar flask of 1 litre capacity and 28 cm internal depth, surrounded by a metal casing, is tightly closed by means of a large cork of about 3 cm thickness. Through the centre of the cork is tightly fitted a medium thin-walled glass or metal tube, 250 mm in length by 33 mm outside diameter. At one side of the cork is inserted a narrow metal inlet tube, the lower end of which is formed into a perforated loop near the bottom of the flask. At the opposite side is a metal tube of T-shape construction and 6 mm internal diameter, intended to afford escape for vapours, and also for introducing volatile fluid into the apparatus. At the back portion of the cork is fitted a control thermometer, the bulb of which extends nearly to the bottom of the flask. The freezing test-tube is of thin glass, about 240 mm in length by 29 mm outside diameter, and fits closely into a larger tube, which is sealed into the cork. In the rubber stopper of the freezing-tube is fitted the standard thermometer. The thermometer is constructed of such length as to enable insertion of the bulb nearly to the bottom of the tube and at the same time allow complete exposure of the scale above the stopper. At the right side of the thermometer a stirring device made of non-corrodible low-conductivity metal is fitted into the stopper through a short section of thin-walled metal tubing. The lower end extends nearly to the bottom of the test-tube, and is provided with a horizontal loop encircling the thermometer. At the left of the thermometer is a freezing-starter attachment, inserted through an opening in the stopper formed by means of a short section of metal tubing. This device consists of a non-corrodible metal rod, at the lower end of which is a 10 mm length opening for the purpose of carrying a small fragment of ice. At one side of the cryoscope is installed an air-drying arrangement, which consists of a Folin absorption bulb inserted through a tightly-fitting stopper and extending nearly to the bottom of a large-sized test-tube. A short section of glass tubing is inserte through a second opening in the stopper and is connected to the vaporisin tube which enters the cryoscope. Sulphuric acid is poured into the dryin tube to a level slightly above the small inner bulb. At the opposite side of th apparatus is arranged a drain tube for the purpose of conducting vapours awa from the operator. By means of a pressure- and suction-pump, dry air may b forced into the apparatus at a suitable rate and the mixed vapours conducte out through the base of a drain tube into the sink. An adjustable lens i mounted in a convenient position in front of the thermometer for the purpos of magnifying the scale.

The revisers have found that it is an advantage to have an additional trap in the form of a small bottle between the sulphuric acid tube and the vaporisin tube which enters the cryoscope, to prevent corrosion due to access of sulphuri acid spray into the apparatus.

The standard Hortvet thermometer and the control thermometer are described in detail on p. 126.

The cryoscope devised by Temple (1937) employs mechanical refrigeratio instead of evaporation of ether to maintain the temperature of the cooling bath. The apparatus gives exactly comparable results to those obtained by th Hortvet cryoscope, and is extremely useful in any laboratory where larg numbers of freezing-point tests are carried out.

Method of carrying out the test

The following description is taken from the A.O.A.C. *Methods of Analysis* 7th edn., p. 237—

Insert the funnel tube into the vertical portion of the T-tube at one side o the apparatus and pour in 400 ml of ether, previously cooled to 10° C or lowe Close the vertical tube by means of a small cork and connect the pressure pump to the inlet tube of the air-drying attachment. Adjust the pump so a to pass air through the apparatus at a moderate rate, as may be judged by th agitation of the sulphuric acid in the drying tube. Continuous vaporisatio of the ether will cause a lowering of the temperature in the flask from the ordinary room temperature to 0° in from 5 to 10 minutes. Continue the temperatur lowering until the control thermometer registers near − 3°. At this stage, b lowering the gauge tube into the ether bath, then closing the top by means o the forefinger and raising to a suitable height, an estimate can be made as t the quantity of ether necessary to pour in for the purpose of restoring th 400 ml volume. When the volume of ether has been adjusted to 400 ml, a additional 10 to 15 ml is sufficient on an average for each succeeding determina tion. Pour into the freezing-tube sufficient water (30 to 35 ml), boiled an cooled to 10° or lower, fairly to submerge the thermometer bulb. Insert th thermometer together with the stirrer and lower the test-tube into the larg tube. A small quantity of alcohol, sufficient to fill the lower space between t two test-tubes, will serve to complete the conduction medium between t freezing-bath and the liquid to be tested.

Keep the stirrer in steady up-and-down motion at a rate of approximat 1 stroke each 1 or 2 seconds, or even at a slower rate, provided the cool proceeds satisfactorily. Maintain a passage of air through the apparatus un the temperature of the cooling-bath reaches − 2·5°, at which time the top the mercury thread in the thermometer usually recedes to a position near the freezing point of water. Maintain the temperature of the cooling-bath at —2·5° and continue the manipulation of the stirrer until a supercooling of sample of 1·0° to 1·2° is observed. As a rule, at this time the liquid will begin to freeze, as may be noted by the rapid rise of the mercury. Manipulate the stirrer slowly and carefully 3 or 4 times as the mercury column approaches its highest point. By means of a suitable light-weight cork mallet, tap the upper end of the thermometer cautiously a number of times until the top of the mercury column remains stationary for at least 1 minute. Observe the exact reading on the thermometer scale, taking necessary precautions to avoid parallax, and estimate to 0·001°. When the observation has been satisfactorily completed, make a duplicate determination; then remove the thermometer and stirrer and empty the water from the freezing-tube.

Rinse the tube with about 25 ml of the sample of milk, cooled to 10° or lower; measure into the tube 30 to 35 ml of milk, or enough fairly to submerge the thermometer bulb, and insert the tube into the apparatus. Maintain the temperature of the cooling-bath at 2·5° below the probable freezing point of the sample. Make the determination on the milk, following the same procedure as that employed in determining the freezing point of water. As a rule, however, it is necessary to start the freezing action in the milk by inserting the freezing starter (which has been kept in contact with ice for several minutes, and in the open end of which has been wedged a fragment of ice) at the time when the mercury column has receded to 1·0° to 1·2° below the probable freezing point. A rapid rise of the mercury results almost immediately. Manipulate the stirrer slowly and carefully two or three times while the mercury approaches its highest point. Complete the adjustment of the mercury column in the same manner as in the preceding determination; then, avoiding parallax, observe the exact reading on the thermometer scale and estimate to 0·001°. The algebraic difference between the average of readings obtained on the water and the reading obtained on the sample of milk represents the freezing-point depression of the milk. Apply necessary correction to the result (i.e. any thermometer corrections).

The determination of the freezing point of sugar solutions is carried out exactly as in the case of milk, i.e. the control thermometer is kept approximately 2·5° below the expected freezing point of the solution, and the solution is allowed to cool 1·0° to 1·2° below its freezing point and is then seeded with ice.

It is advisable to repeat the stirring and tapping of the thermometer, particularly with milk, when the mercury has reached its highest point; the risk of recording a false freezing point is thereby minimised.

The most efficient method of seeding the sample with ice is to cool the freezing starter tube in contact with ice and then, immediately before introducing it into the sample, it is stroked, open end foremost, across the surface of a dry piece of ice, thereby readily introducing fragments of ice into the open end of the tube.

$$\text{Per cent added water} = \frac{100\,(\text{F.P.} - \text{F.P.}')}{\text{F.P.}}$$

where F.P. is the freezing point of the original unadulterated milk and F.P.′ the observed freezing point of the adulterated sample.

B.S. specification and technique

The following technique has been prepared in draft form by the British Standards Institution.

1.1. *Definition.* The freezing-point depression △ is the freezing point of pure water minus the freezing point of milk expressed in °C.

1.2. *Apparatus.*

1.2.1. *Cryoscope*[1].

A cylindrical Dewar flask, 75 mm in internal diameter and 280 mm in depth internally, surrounded by a metal casing, is tightly closed by means of a large cork of about 30 mm thickness. Through the centre of the cork is tightly fitted a nickel-plated brass test tube, 22 s.w.g. thick, 250 ± 2 mm in length and 33 ± 0·1 mm in outside diameter. The freezing test tube fits into this brass tube and is of glass 0·75 to 1·00 mm thick, 240 ± 2 mm in length and 29 ± 1 mm in outside diameter. Four holes are bored equidistantly from the centre of the cork and from each other to accommodate tightly the following parts of the apparatus which protrude into the Dewar flask:

(*a*) A narrow inlet tube of acid-resisting metal the lower end of which is formed into a perforated loop near the bottom of the flask and through which air is forced through the ether in the Dewar flask.

(*b*) A metal tube at the opposite side from (*a*) of T-shape construction and 6 mm internal diameter, intended to afford escape for vapours, for introducing ether into the apparatus, and for inserting a glass gauge tube marked to indicate the ether level when 500 ml have been introduced into the Dewar flask of the assembled apparatus.

(*c*) A control thermometer the bulb of which extends nearly to the bottom of the Dewar flask.

(*d*) A metal tube 25 cm in length, having an external diameter of 6·5 mm and an internal diameter of 4·5 mm, the bottom being closed to accommodate ice for starting the freezing of liquids in the freezing tube. Into this tube passes a metal rod 27 cm in length and 3 mm in diameter fitted at its upper end with a suitable plastic handle 2 cm in length and 5 mm in thickness. The bottom end of the rod is bored centrally for a distance of 1·5 cm to form a tube of 2 mm internal diameter, an escape hole of the same diameter being bored at right angles through the side of the rod to meet the top of this short tube. The bottom end of the tube thus formed is sharpened and segmented into four segments by longitudinal cuts extending about 2 mm up the tube so as to facilitate cutting into the ice in the starting tube, and extracting particles suitable for starting the freezing of liquids when inserted into the freezing test tube.

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[1] The modified cryoscope incorporating an automatic cooling-unit devised by Temple (*Analyst*, 1937, **62**, 709) at the National Institute for Research in Dairying, may be used in place of the Hortvet cryoscope. Its use makes for speed and convenience, and gives results which are in close agreement with those obtained in the Hortvet apparatus, provided that the motors are switched off from the time of seeding until the final reading is taken.

The freezing test tube is fitted with a rubber bung[1] through which pass the following—

(*e*) A Hortvet thermometer which shall be inserted co-axially, so that the bottom of the bulb is approximately 15 mm from the bottom of the freezing test tube.

(*f*) A thin-walled metal tube approximately 25 mm in length and 4 mm in diameter through which loosely passes the stirring device made of non-corrodible low conductivity metal. The lower end of the stirrer extends nearly to the bottom of the test tube and is provided with a horizontal loop encircling the thermometer. The stirrer may be actuated manually or mechanically.

(*g*) A thin-walled metal tube approximately 25 mm in length and 4 mm in diameter through which the freezing starter (see (*d*)) may be inserted at the appropriate time.

NOTE. Where a number of successive determinations is to be made it is convenient to add to the T-tube (see (*b*)) a U-tube measuring gauge with a tap funnel. The glass tubing of the gauge should not be of more than 4·8 mm external diameter in order to allow free passage of ether vapour through the T-tube. The U-tube shall be marked to indicate the ether level when 500 ml have been introduced into the Dewar flask in the assembled apparatus. Adjustment and reading of the ether level should be made after stopping the air current and after removal of any air bubbles in the gauge by the addition of a small quantity of ether through the tap funnel.

At one side of the cryoscope is installed an air-drying arrangement which consists of a Folin absorption bulb inserted through a tightly fitting stopper and extending nearly to the bottom of a large-sized test tube. A short section of glass tubing is inserted through a second opening in the stopper and is connected to the vaporising tube which enters the cryoscope. Sulphuric acid is poured into the drying tube to a level slightly above the small inner bulb.

NOTE. Sulphuric acid may cause corrosion of the perforated metal loop. This can be avoided by first passing the air over calcium chloride in a drying tower and then bubbling it through liquid paraffin placed in the tube intended for sulphuric acid.

At the opposite side of the apparatus is arranged a drain tube for the purpose of conducting vapours away from the operator. Dry air is passed through the apparatus at a suitable rate and the mixed vapours conducted through the base of the drain tube, preferably to the outside atmosphere.

A lens system shall be used in reading the thermometer. It shall be so designed and mounted as to avoid parallax.

1.2.2. *Hortvet thermometer.* The Hortvet thermometer shall be a solid stem thermometer and shall be standardised by the National Physical Laboratory or equivalent institution to an accuracy of at least 0·002° C on the International Temperature Scale. The temperatures at which standardisation is carried out shall include values close to + 0·100°, + 0·040°, + 0·020°, 0·000°, — 0·020°, — 0·500°, — 0·520°, — 0·540° and — 0·560° C.

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[1] A plastic stopper may be used in lieu.

If a thermometer standardised in this manner is not available and the necessity arises to determine the freezing-point depression of a milk sample, the method of standardisation outlined in Appendix D [of the Specification] may be adopted as an emergency measure.

1.2.3. *Control thermometer.* The control thermometer shall be a solid-stem thermometer which shall be standardised by the National Physical Laboratory or equivalent institution.

1.3. *Procedure.* (*a*) *Water.* Insert the funnel tube into the vertical limb of the T-tube and pour in 500 ml of ether cooled to 10° C or lower. Close the vertical limb of the T-tube and pass air through the apparatus at a moderate rate. Continuous vaporisation of the ether will cause a lowering of the temperature in the flask from ordinary room temperature to 0° C in five to ten minutes. Continue until the control thermometer registers approximately — 3° C. At this stage, by lowering the gauge tube into the ether bath, then closing the top by means of the forefinger and raising to a suitable height, an estimate can be made as to the quantity of ether necessary to be added for the purpose of restoring the 500 ml volume. When the volume of ether has been originally adjusted to 500 ml an additional 10 to 15 ml is generally sufficient for each succeeding determination.

Insert the thermometer, together with the stirrer, into the freezing test tube, having poured into the tube sufficient distilled water (recently boiled and cooled), to bring the level of the water to approximately 12 mm above the top of the bulb of the thermometer. Lower the test tube into the larger tube. Pour a small quantity of alcohol into the metal test tube to form a conducting medium between the freezing bath and the inner freezing-point tube. The quantity used shall be sufficient to ensure that the level is above the level of water in the freezing point tube when this is placed in position. Commence stirring and maintain the stirrer in steady up-and-down motion at a rate of approximately one stroke each 1 to 2 seconds or even at a slower rate, providing the cooling proceeds satisfactorily.

Maintain the passage of air through the apparatus until the temperature of the cooling bath reaches — 2·5° C, at which time the top of the mercury thread in the thermometer usually recedes to a position near the freezing point of water. Maintain the temperature of the cooling bath at — 2·5° C and continue the manipulation of the stirrer until a super-cooling of the water of 1·0 to 1·2° C is observed. As a rule, at this time the water will begin to freeze, as may be noted by the rapid rise of the mercury. If the water does not freeze spontaneously, insert the freezing starter tube with a fragment of ice. The tube shall previously have been in contact with ice for several minutes. Stir at the prescribed rate until the mercury thread rises. Immediately remove the freezing starter tube and cease stirring. After about 90 seconds the apparent highest point should be reached. Then stir three times at normal speed, tap the thermometer stem seven times at the level of the top of the mercury thread and read, taking the necessary precautions to avoid parallax and estimate to 0·001° C. After an interval of 30 seconds after this reading, stir again three times followed by tapping seven times as before. Read. Repeat these operations a third time and record the final reading. All three readings shall be in close agreement.

When the observation has been satisfactorily completed, make a duplicate determination, then remove the thermometer and stirrer and empty the water from the freezing tube.

(*b*) *Milk.* Rinse the tube, thermometer and stirrer with a portion of the sample of milk, preferably cooled to 10° C or lower. Insert the thermometer, together with the stirrer, into the freezing test tube, having poured into the tube a sufficient portion of the milk sample to bring the level of the milk to approximately 12 mm above the top of the thermometer bulb. Lower the test tube into the larger tube. Commence stirring and maintain the stirrer in steady up-and-down motion at a rate of approximately one stroke each one to two seconds or even at a slower rate, providing the cooling proceeds satisfactorily.

Maintain the passage of air through the apparatus until the temperature of the cooling bath reaches — 3° C. Maintain the temperature of the cooling bath at — 3° C and continue the manipulation of the stirrer until the mercury column in the Hortvet thermometer has receded to — 1·65° C.

Insert the freezing starter tube which has been in contact with ice for several minutes and which holds a fragment of ice. Stir at the prescribed rate until the mercury thread rises. Immediately remove the freezing starter tube and cease stirring. The apparent highest point should be reached in approximately 90 seconds. Then stir three times at normal speed, tap the thermometer stem seven times at the level of the top of the mercury thread and read, taking the necessary precautions to avoid parallax, and estimate to 0·001° C. After an interval of 30 seconds after this reading, stir again three times followed by tapping seven times as before. Read. Repeat these operations a third time and record the final reading.

The difference between the first and second readings may exceed 0·005° C, but will not usually exceed 0·01° C. If the difference between the second and third readings exceeds 0·003° C the results shall be disregarded and the test recommenced.

When the observation has been satisfactorily completed, make a duplicate determination.

It is not essential to repeat the determination of the water value after each milk determination when a series is being carried out, but frequent checking of the water value is necessary to follow any alteration in the zero.

Determine the titratable acidity of the milk sample in accordance with B.S. No. 1741—1951. With fresh milk it lies normally between 0·12 and 0·18 per cent (expressed as lactic acid w/v).

1.4. *Calculation of depression* (△) *of the freezing point of milk.* To the thermometer readings for water and milk add the appropriate corrections obtained by linear interpolation from the values given in the thermometer certificate, and subtract the freezing point of the milk sample from that of the water.

Expression and Interpretation of Results

The average freezing-point depression of genuine milk is approximately 0·545° C, but with milk from individual cows and also with small bulks of mixed milk there may be considerable variation from this figure. In practice, the freezing-point depression of quantities of 200 gallons is unlikely to be less than 0·540° C, while milk from individual cows is unlikely to have freezing-point depressions of less than 0·530° C.

The development of acidity in a sample of milk causes an increase in the freezing-point depression which might mask, partially or completely, the contrary effect of added water. It is, therefore, desirable to determine the freezing-point while the milk is fresh, and essential that every report on the freezing-point test should be accompanied by a statement of the titratable acidity of the sample at the time of testing determined in accordance with B.S. No. 1741. When the milk is no longer fresh, but the degree of souring is not excessive, a good approximation to the freezing-point depression of the milk when fresh can be obtained by the application of a correcting factor to the observed freezing-point depression.[1]

Where the titratable acidity exceeds 0·18 per cent expressed as lactic acid w/v, but does not exceed 0·30 per cent, a correcting factor of 0·0034° C for each 0·01 per cent of lactic acid shall be applied according to the formula—

$$\triangle \text{ corrected} = \triangle - 0{\cdot}34\ (\text{percentage lactic acid} - 0{\cdot}18)$$

Where the titratable acidity exceeds 0·30 per cent, the freezing-point test should not be applied.

The proportion of added water in any sample of milk is given by the following—

$$W = \frac{(\triangle_1 - \triangle)}{\triangle_1} \times (100 - \text{T.S.})$$

where W = the percentage of added water by weight;
$\triangle_1$ = the freezing point depression of the genuine milk;
$\triangle$ = the observed freezing-point depression after adulteration corrected, if necessary, for the acidity of the milk, and
T.S. = the percentage of total solids.

As the original freezing-point depression of any sample of adulterated milk is usually unknown, it is necessary to adopt a figure for calculation of the proportion of added water—

(*a*) For milk known to have been mixed in quantities of 200 gallons or more	0·540° C
(*b*) For other milks	0·530° C

The result obtained by substitution of one of these values for $\triangle_1$ in the above formulae should be reported as "*minimum* percentage of added water", stating the arbitrary figures chosen and the formula used. (See also pp. 144 to 147).

In view of the variations in the freezing point of genuine milk, greater depressions than the limits used in the calculation do not necessarily indicate that the milk is free from added water.

Where a series of results on bulk milk from any particular source is available, large variations in the freezing-point depression from day to day are indicative of adulteration.

(5) DETERMINATION OF THE ACIDITY OF MILK

The term "acidity" is used somewhat loosely in the dairy industry and usually means the "titratable acidity", which is a measure of the buffering power of milk from pH 6·6 (that of milk) to pH 8·4 (the end-point of phenolphthalein in milk), and in unsoured milk has nothing to do with lactic acid. It is correlated to some extent with solids-not-fat (cf. p. 153) and more especially with the contents of protein and phosphates. When milk sours, lactic acid is formed and the pH value falls, ultimately reaching a value of about 4·2 or even lower with the later growth of lactobacilli. The titratable acidity then includes both the natural buffer value of the sweet milk and the acid produced by the lactic fermentation.

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[1] Report of a Sub-Committee of the Scientific Advisory Committee, Department of Health for Scotland, Edinburgh, H.M. Stationery Office, 1946.

If the *true* lactic acid content of milk is required, special chemical methods must be used.

For a further discussion of the subject see Davis (1950a).

Titratable acidity

The acidity of milk is determined by titration with alkali, using phenolphthalein as indicator; 10 ml are placed in a small porcelain dish or beaker, and 1·0 ml of phenolphthalein solution (0·5 g per 100 ml of 50 per cent alcohol) is added and then titrated with 0·1 N NaOH or strontia until a faint pink colour, equal to that produced by the addition of 1 drop of a 0·01 per cent solution of rosaniline acetate in 96 per cent alcohol to a similar volume of milk, is obtained. This procedure should not be varied. The figures thus obtained are termed by Richmond and Huish " acidity (R.S.) ", the letters R.S. indicating rosaniline standard.

Generally speaking, about 1·7 ml to 2·0 ml of 0·1 N alkali are required for fresh milk; each cubic centimetre of N alkali used per litre of milk is called 1° of acidity; hence a milk requiring 2 ml of 0·1 N solution for 10 ml will have 20° of acidity.

Pizer (1936) has examined the relationship of the visible end-point with phenolphthalein to the pH value, and has shown that it varies with the concentration of the indicator. At the concentration of indicator used above, 0·05 g per 100 ml of milk, the colour of the indicator becomes visible at pH 8·4. When the concentration of indicator was 0·005 g per 100 ml, colour only became visible at pH 8·8 to 8·9, and with less indicator only at pH 9·1.

The acidity of milk to phenolphthalein is due partly to mono- and dibasic phosphates and partly to the dissolved carbonic acid. Although freshly-drawn milk has a distinct acidity, it is probable that lactic acid is absent, and the acidity (of fresh milk at least) should therefore not be expressed in terms of this acid. Should it, however, be desired to express the acidity of sour or other milks in terms of lactic acid, then 1 ml 0·1 N alkali = 0·009 g of lactic acid; or degrees acidity × 0·09 = lactic acid per cent wt/vol.

Storch used a solution of lime-water containing solid lime as a standard alkaline solution; this remains constant in composition and is nearly one-twentieth normal. The strength of the solution remains constant, as, if any of the lime is removed by carbon dioxide, more is dissolved, and it is little affected by ordinary variations in temperature. The use of this solution in dairy laboratories is recommended, as the only precaution necessary is to have an excess of lime in the bottle.

M'Creath employed a caustic soda solution of such a strength that each ml = 0·01 g of lactic acid. Ten ml of milk, after the addition of phenolphthalein solution, are titrated, and the number of ml used divided by 10 gives the acidity in terms of lactic acid.

Soxhlet and Henkel used 0·25 N NaOH solution, and they also expressed the acidity as degrees. One Soxhlet-Henkel (S.H.) degree = the number of millilitres of 0·25 N alkali used per 100 ml of milk. This degree is consequently 2·5 times larger than the degree of the R.S. standard and may therefore cause confusion in comparing results.

Degrees S.H. × 0·023 = percentage lactic acid

A modified determination of the acidity can be carried out by using neutral litmus paper instead of phenolphthalein. Fresh milk is practically neutral to litmus, and the acidity can be titrated with fair accuracy, although the end-point is not sharp. The acidity thus determined may be considered as an approximation of the lactic acid present. The following figures obtained by Richmond on sour milks will show the vast difference in the two sets of results, the figures in both cases being calculated to lactic acid—

	I	II	III	IV	V
Acidity (to phenolphthalein)	1·24	1·89	1·82	1·52	1·32
Acidity (to litmus paper) ..	0·65	1·14	1·28	0·86	0·56

Another approximation of the amount of lactic acid present may be arrived at by distilling some of the milk into a known amount of 0·1 N alkali, titrating back with 0·1 N acid, using litmus paper as indicator, and subtracting the volatile acidity from the total acidity to litmus; the non-volatile acidity is taken as lactic acid.

It will be seen from the above that a large number of methods are in existence for the estimation of acidity, and there is certainly room for standardisation of technique. Davis and Sadek (1942) have discussed the more important factors involved in the test, and recommend that 20 ml of milk be taken, 2 ml 0·5 per cent phenolphthalein solution be used as indicator, and that the end-point be taken as the faint greyish pink colour which lasts for 5 seconds. They also state that a control sample shall be used for judging this end-point and that the temperature of the milk shall be between 55° and 75° F (12·8°–23·9° C). N/9 sodium hydroxide is used.

The British Standard (No. 1741—1951) method is as follows:

APPARATUS. 25 ml Class A or B[1] burette with soda-lime guard tube.
Class A or B pipette[2] to deliver 10 ml.
Pipettes or burettes to deliver 1 ml.
Hemispherical white porcelain basins of approximately 60 ml capacity.
Glass stirring rods.

REAGENTS. The reagents used shall be of recognised analytical reagent quality.
N/9 sodium hydroxide solution, carbonate-free.
Phenolphthalein indicator solution. Dissolve 1 g of phenolphthalein in 110 ml of ethyl alcohol (95/96 per cent v/v). Add approximately decinormal sodium hydroxide solution until one drop gives a faint pink coloration. Make up to 200 ml with distilled water.
Rosaniline acetate solution. Stock solution. Solution A. Dissolve 0·12 g of rosaniline acetate in approximately 50 ml of ethyl alcohol

[1] B.S. 846, "Burettes and bulb burettes."
[2] B.S. 700, "Graduated pipettes and straight pipettes."

(95/96 per cent v/v) containing 0·5 ml of glacial acetic acid. Make up to 100 ml with ethyl alcohol.

Bench solution. Solution B. Dilute 1 ml of Solution A to 500 ml with a mixture of ethyl alcohol (95/96 per cent v/v) and distilled water in equal proportions by volume.

Note.—Solutions A and B should be stored in the dark in bottles securely stoppered with rubber bungs.

PROCEDURE. Thoroughly mix the sample of milk, avoiding incorporation of air bubbles and transfer 10 ml to each of two porcelain basins. Prepare a blank from one 10-ml portion by stirring in 1 ml of Solution B of rosaniline acetate.

Add 1 ml of phenolphthalein indicator solution to the other 10 ml of milk, followed by the rapid addition of about 1 ml of N/9 sodium hydroxide solution and continue to add drop by drop until by comparison the colour matches the pink tint of the blank. Stir vigorously throughout. The time taken for the complete titration shall not exceed 20 seconds.

The titration shall be made in a north light or under illumination from a daylight lamp complying with B.S. 950[1].

Express the acidity in terms of " lactic acid ", g/100 ml of milk, by dividing by ten the number of millilitres of N/9 sodium hydroxide solution required.

Lactic acid (as such)

Lampitt and Bogod (1930) describe a method for the determination of lactic acid in milk, etc., depending upon the oxidation of lactic acid in a clarified solution to acetaldehyde by means of potassium permanganate; the acetaldehyde is distilled into standard sodium bisulphite solution, the excess of sodium bisulphite being afterwards titrated with 0·01 N iodine solution.

Hillig (1937) describes a colorimetric method for the determination of lactic acid in milk, etc., which makes use of the yellow colour produced in the reaction between ferric chloride and lactic acid. Davidson (1949) has described a quick method for determining lactic acid in milk and unsweetened milk products. The acetaldehyde produced by oxidation by sulphuric acid in the presence of copper sulphate is allowed to develop a purple colour with *p*-hydroxydiphenyl and the intensity of colour measured with a photoelectric absorptiometer. An average recovery of 100 per cent (96 to 103) is claimed.

Methods for the determination of lactic acid in milk have also been studied by Ling (1951). Proteins and citrate are precipitated by sodium hydroxide and zinc sulphate in the presence of barium chloride, the colour being measured by any one of three well-known methods. It is stated that by means of standard curves based on the estimations of known amounts of lactic acid in milk an accuracy comparable with that of the *p*-hydroxydiphenyl method can be obtained.

Ling finds that the apparent concentration of lactic acid in fresh milk is about 0·003 per cent and its value may increase appreciably as lactation progresses.

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[1] B.S. 950, " Artificial daylight fittings for colour matching."

CHAPTER 16

THE DETERMINATION OF FAT IN MILK

More attention has perhaps been paid to the determination of fat in milk than to that of any other constituent. The methods are very numerous, and may be divided conveniently into three classes—

(*a*) *Gravimetric methods*, in which the fat is separated from the milk by a suitable solvent, and weighed after evaporation of the solvent. The chief methods which can be employed are: the Röse-Gottlieb process, the Werner-Schmid process, the Adams process and the maceration process; the last is described under "Analysis of sour milk" (p. 437). Hostettler (1947) has critically reviewed many methods for the determination of fat. He concludes that none gives a true value for pure fat, as lecithin and other substances are extracted. The Röse-Gottlieb is considered to be the best.

(*b*) *Volumetric methods*, in which the fat is separated from the milk by suitable means, and its volume measured. The chief methods of this type are: the Gerber process, the Leffmann-Beam process, and the Babcock process. Schwarz and Mumm (1947) find that butyrometers give the closest agreement with the Röse-Gottlieb when the contents are kept at 70° C for 30 min. before centrifuging. Martin *et al.* (1942) and Hansen and Deane (1942) have discussed the factors affecting the accuracy of the Babcock and other American tests for fat. Lucas and Trout (1947) have collected the best modifications of the Babcock test into one procedure for homogenised milk. A mean loss of only 0·014 per cent due to homogenisation was recorded. Trout and Lucas (1947) have compared five rapid fat tests for homogenised milk. The Gerber test was most reproducible and gave the same results for ordinary and homogenised milks, which were about 0·1 per cent higher than by the Mojonnier method. The Babcock was nearer the Mojonnier in its results. Koeder (1940) has studied the sources of error in the Gerber test and his figures indicated that the error should be within 0·05 per cent in 88 and within 0·1 per cent in 99·7 per cent of tests.

(*c*) *Indirect methods*, in which the amount of fat is deduced from the determination of some physical property: for example, the refractive index of an ethereal solution of the fat; or from the specific gravity and total solids by means of the "milk scale".

(A) GRAVIMETRIC METHODS

The Röse-Gottlieb, the Werner-Schmid, and the Adams processes are described in the following pages. The Röse-Gottlieb process is preferred by many workers, chiefly because the fat is not submitted to any drastic pretreatment with acid or by heating, and secondly because the process is fairly rapid and gives very clean separations of the fat solvents from the aqueous layer. The three processes described here and the maceration process are, however, all capable of a similar degree of accuracy.

The Röse-Gottlieb process

The following description of a modification of the process is taken from Report No. 1 of the Milk Products Sub-Committee to the Standing Committee on Uniformity of Analytical Methods of the Society of Public Analysts (Soc. Publ. Anal., 1927).

The method is primarily used for the determination of fat in condensed milk (sweetened or unsweetened), but it is equally available for fresh or sour milk, 10 g being used in place of the 2 to 2·5 g of condensed milk and the 8 ml of warm water.

It is essential that the directions should be followed implicitly, and if any clots appear on addition of ammonia and alcohol they should be broken up by mixing before ether is added, or the fat will not be completely extracted. The corks used in the extraction apparatus should be previously solvent-extracted and should be moistened with water immediately before use. The extraction apparatus may consist of a stoppered cylinder or a light, flat-bottomed, stoppered tube about 7 in. by 1 in., or an apparatus of the type due to Eichloff and Grimmes (Fig. 2).

The method as described for condensed milk is as follows—

REAGENTS. Concentrated ammonia solution, nominal 0·880.
Alcohol or industrial methylated spirit, 95 per cent by volume.
Ether (methylated), sp. gr., 0·720.
Petroleum spirit, boiling between 40° C and 60° C.
These reagents should leave no appreciable residue on evaporation.

PROCEDURE. Transfer to a suitable apparatus from 2 to 2·25 g, accurately weighed, of the well-mixed sample; add 8 ml of warm water and mix well; cool; add 1 ml of concentrated ammonia solution, mix, add 10 ml of alcohol and again mix. Add 25 ml of ether and shake vigorously for 1 minute; add 25 ml of petroleum spirit and again shake vigorously for 30 seconds. Allow the liquids to stand for not less than half-an-hour, until the ethereal layer is perfectly clear, or centrifuge at a low speed. Transfer the ethereal layer to a suitable flask. To the milk residue add 5 ml of ether, and transfer without further shaking; repeat this operation in the same manner with a further 5 ml of ether. Add 0·5 ml of alcohol, and repeat the extraction with 25 ml of ether and 25 ml of petroleum spirit, as before, shaking vigorously for one minute after the addition of the ether and for 30 sec. after the addition of the petroleum spirit. As before, allow the ethereal layer to separate completely and transfer to the flask. Repeat the extraction once more with alcohol, ether and petroleum spirit in the same manner.

Cautiously distil the solvents from the flask and dry the residual fat at 98° to 100° C to constant weight, taking the ordinary precautions to remove all traces of volatile solvent.

Completely extract the fat from the flask by repeated washings with petroleum spirit, allowing any sediment to settle before each decantation. Finally dry the flask at 98° to 100° C. The difference in weights before and after the petroleum spirit extractions is the weight of fat contained in the quantity of condensed milk taken.

Make a blank determination, using the specified quantities of reagents, and distilled water in place of the milk, and deduct the figure found, if any, from the weight of fat obtained.

Muers and House (1949) have found that the use of ether which contains peroxide can yield high results by the Röse-Gottlieb or Werner-Schmidt method, such errors amounting to as much as 2 per cent of the fat figure in some cases. The ether can be purified by the following procedure. Strips of zinc–copper couple are prepared by immersing zinc foil in acidified copper sulphate solution for about a minute and are then transferred, while still wet, to the ether container. Seven or eight 50 × 5 cm strips in 5 gallons of ether gave satisfactory results, and it was not necessary to filter or distil the treated ether before use.

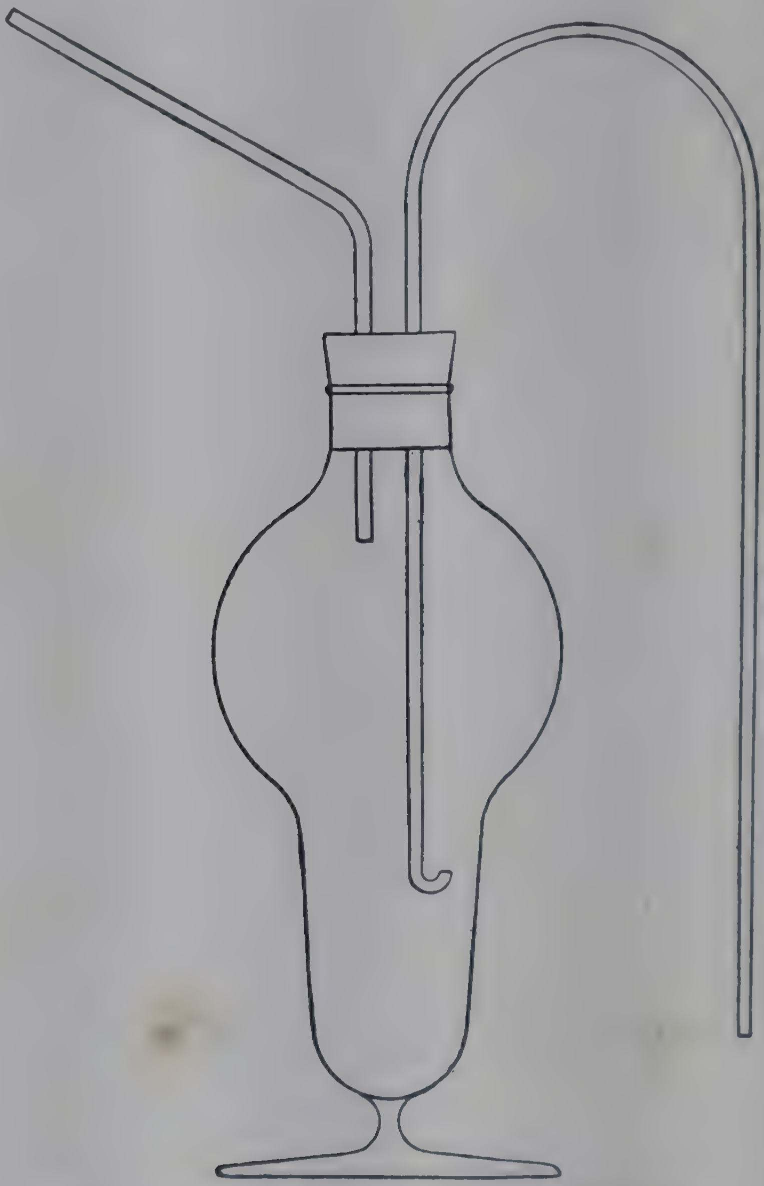

Fig. 2—Eichloff and Grimmes' apparatus

(E. R. Bolton) (by permission of Messrs J. & A. Churchill)

Modified method

A useful modification of the above method, which has been used for some years by the revisers, is the following. It is more rapid, requires a smaller quantity of solvents, and yields results comparable with those obtained by the Röse-Gottlieb method. (The usual Röse-Gottlieb is normally used in this country for legal purposes.)

Ten ml of the well-mixed milk are weighed into a Stokes tube; 1 ml concentrated ammonia solution is added and the liquids well mixed; 10 ml alcohol are then added and the liquids again well mixed. After the addition of 19 ml of ether the tube is shaken vigorously for one minute, 19 ml petroleum ether are added and the vigorous shaking repeated. The tube is then centrifuged for fifteen minutes in a Gerber machine, and the total volume of the ethereal layer recorded. The greater part of this layer is then blown into a tared flask, the volume of the residual ether layer recorded, and the pipette washed into the flask with a little ether. The ethers are distilled off and the flask and contents taken to constant weight at 100° C.

The flask may be extracted with petroleum ether, as in the original Röse-Gottlieb method, and a correction made for any non-fatty material, but the amount of the latter extracted under the above conditions is usually negligible.

$$\text{Percentage fat} = \frac{F \times E_1 \times 100}{W \times E_2}$$

where F is the weight of fat extracted, E_1 the total ether volume, E_2 the ether volume used, and W the weight of milk taken.

The process is applicable to dried milk, condensed milk, cream, and ice-cream.

The Werner-Schmid method

This process differs from the others in that hydrochloric acid is used to dissolve the casein, the liberated fat afterwards being extracted with ether. It is the insoluble casein which hinders the separation of fat in milk and thereby prevents theoretical results being obtained when ether alone is used for extraction purposes.

Ten g of milk are weighed into a Schmid or Stokes tube, graduated in tenths of a ml; 10 ml of strong hydrochloric acid are then added and the tube is immersed in a boiling water-bath and occasionally shaken until the mixture has become brown and the casein has dissolved. The tube is then cooled completely, 30 ml of ether added, and the tube corked; shake at first gently, loosening the cork to release pressure, and then mix thoroughly, but do not shake vigorously. Allow to stand until separation is complete, assisting the process by gently rotating the tube, and then read off the volume of ether, adding on three-quarters the volume of any fluffy-looking stratum, between the ethereal and aqueous layers, to the ether volume. This fluffy layer, after separation is complete, should not amount to more than about 1·0 ml. Pipette as much of the clear ethereal layer as is conveniently possible into a tared flask; note the volume of the ethereal layer remaining in the tube, and wash the pipette with a little fresh ether into the flask. Distil off the ether, dry at 100° C, and weigh the residue. The flask may then be extracted with petroleum ether, reweighed, and the weight of any non-fatty matter deducted from the first weighing.

Stokes later introduced a new form of tube, in which the middle portion is narrowed for greater accuracy of measurement of the ethereal layer.

An obvious modification of the Werner-Schmid process is the complete extraction of the acid layer with ether, which may readily be carried out as follows. Ten grams of milk are weighed into a small beaker to which is added 10 ml of concentrated hydrochloric acid, the beaker is covered with a watch-glass, and heated on a gauze over a very small flame until the solution is brown and the fat has separated (care being taken not to overheat the mixture). The contents of the beaker are allowed to cool and are then washed into a separator with ether and a few millilitres of water. The solution is extracted three times with ether, care being taken to prevent the formation of heavy emulsions; in this connection it will be found helpful if the aqueous layer is kept as small in bulk as possible. The combined ethereal extracts are then washed with water, at first gently and then more vigorously, until the washings are free from chlorides. The ethereal layer is then filtered into a tared flask, the separator and filter washed with fresh ether, the ether evaporated, and the residue dried at 100° C and weighed. The flask may be re-extracted with petroleum ether to obtain the weight of any non-fatty matter, but this should be negligible if the ether layers are properly washed.

The Adams method

Dr. M. A. Adams, a former Public Analyst for Kent, was led to devise this method from his observation that when milk was dropped on blotting-paper, it spread out to a much greater extent than was possible in a basin, flask, or even on a flat surface of glass, and he was of opinion that extraction of fat by ether would then be much more complete.

As originally designed, the method was as follows. Strips of white blotting-paper, " Mill 428 ", 2½ in. wide by 22 in. long, were coiled up loosely and held by having a brass ring slipped over them. These were dried at 100° C to constant weight, the weighings being performed in a weighing-bottle to prevent absorption of moisture from the atmosphere. Five ml of milk were pipetted out into a small beaker and the weight noted; one of the coils was dropped in, and the milk absorbed as completely as possible by the blotting-paper. When absorption was complete, the coil was carefully removed and placed, dry end downwards, on a glass plate, the beaker being again weighed and the quantity of milk taken up by the coil found from the difference of the two weights. The coil was transferred to a drying oven at 100° C, and left therein till it ceased to lose weight. The original method was thus available for the determination of total solids as well as of fat. The dry coil was placed in a Soxhlet extractor[1] and the fat separated from the solids-not-fat by ether. The total extract, after evaporation of the ether and drying at 100° C, was regarded as fat.

Allen and Chattaway modified this method by rolling up a piece of string with the coil, to keep the layers of paper from touching each other; they also wrapped a piece of filter-paper around it, in order that no milk might escape when a weighed quantity was poured thereon.

Thomson also modified it by hanging up a strip by one end and running the milk on to it from a pipette, afterwards noting the weight of milk delivered by the pipette. He preferred the use of filter-paper instead of the blotting-paper recommended by Adams.

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[1] The Soxhlet extractor was really devised by Szombathy; it was described by Soxhlet, and due credit was given by him to the inventor. The apparatus is, however, always known by Soxhlet's name.

Vieth, immediately after the publication of the method, subjected it to an exhaustive test, and criticised it somewhat severely. He showed that blotting-paper contained matter soluble in ether, and that, as Adams had ignored this, the fat estimations made by Adams were too high; he also showed that the substance in filter-paper soluble in ether was extracted with comparative slowness by this solvent. Faber later showed the same thing.

Disregarding these criticisms, the Milk Committee appointed by the Society of Public Analysts recommended the adoption of this method by their members; it was indeed recommended that papers should be previously extracted, but nothing was said of the difficulty of completely removing the matter soluble in ether, it being implied that twelve siphonings were sufficient to effect this. The Milk Committee's recommendation was adopted at a general meeting of the Society, and the Adams method thus gained quasi-official recognition, though its use for determining total solids was abandoned.

Notwithstanding the specific recommendation of the Milk Committee, however, it became the general practice to use unextracted coils, making a deduction, from the weight of total extract, of the weight of the extract obtained from a coil when extracted alone for the same length of time.

Richmond showed that this last modification was not free from error; the matter soluble in ether was found to consist chiefly of a calcium salt of resinous acids, which was only of limited solubility in ether; the acids themselves were much more soluble, and when these were liberated by other acids—even the small amount of acid found in milk—a greater extract was obtained in a given time. As the time usually allowed for extraction ($1\frac{1}{2}$ hours) was not sufficient to remove the whole of the soluble matter from the blotting-paper—as much as ten hours being necessary—it followed that the matter extracted by ether from the coil was greater when a milk (containing small amounts of acid) was placed on a coil than when the coil was extracted alone. The difference was represented by the amount of resinous acids equivalent to the acidity of the milk, and was naturally not constant.

Richmond found that alcohol extracted the coils completely—a fact also noted almost simultaneously by Soxhlet—but he preferred the use of alcohol containing 10 per cent of acetic acid. Ether containing acetic acid was also efficacious.

A " fat-free " paper is on the market, and this is very generally employed. This " fat-free " paper gives a small ether extract, consisting chiefly of loose fibres; such paper was at first remarkably free from extract, but later batches were found to contain quite an appreciable amount. It is preferable for the analyst to extract his own papers for one or two hours with " acid alcohol ".

Waller and Liebermann objected to the use of ether as a solvent for fat, on the ground that substances other than fat in milk were ether-soluble. Richmond found, however, that, provided the coil is well dried previous to extraction, chloroform, benzene, and petroleum ether give the same results as anhydrous ether; ordinary ether, which contains small amounts of water and alcohol, gives slightly higher results, especially if the coils are allowed merely to air-dry. The error introduced by the use of ordinary ether is small, and very frequently neglected.

Attempts have been made to substitute other substances for the blotting-paper; Abraham, indeed, before Adams published his method, had used " Parker's fibre lint ". Wiley and also Johnstone tried asbestos paper, but the results were not satisfactory.

The action of the blotting-paper appears to be slightly different from that supposed by Adams. Undoubtedly he was correct in supposing that the milk was spread out over a large surface; Richmond's experiments showed that when milk was filtered through blotting-paper the filtrate contained the solids-not-fat, but only a small amount of fat. This view, however, was found by Vieth not to be entirely correct; he found that a portion of the casein was also removed from the milk by blotting-paper. When milk is spread on blotting-paper, the portion which soaks in consists of the whole of the water, milk-sugar and salts, and a considerable proportion of the proteins, together with a small amount of fat; the bulk of the fat, together with a proportion of the casein, is left on the surface, and is very easily extracted by the ether. If the fat globules have been broken up by a " homogeniser ", the extraction is not complete.

Modified method

The following mode of procedure is a slight modification of that recommended by Richmond.

A number of " fat-free " Adams strips, equivalent to the number of samples to be examined, are hung up by means of pins or clamps (letter-clips are very serviceable). The strip is held by the hand at the free end, which is turned up about ½ in., so that the paper is nearly horizontal; 5 ml of milk are then run on from a pipette, in a slow stream, spreading the milk well over the paper. The weight of the milk delivered by the pipette should be noted, care being taken that it is delivered into the weighing vessel at the same rate and in the same manner as it was run on to the paper (this may be conveniently done by pipetting the 5 ml for the total solids in the same way, the weight of the sample taken for total solids then being equal to the amount taken for the Adams process). The papers are allowed to hang up, away from contact with any other object and in a position free from dust, until apparently dry; flies must not be permitted to settle on the surface of the paper, as they consume portions of the fat.

When the strips are dry enough to handle, they should be rolled up in loose coils of such diameter that they will go easily into the Soxhlet extractor (¾ to 1 in.), and these should be temporarily fastened by means of a brass ring, a piece of cotton, or a small pin. A pencilled number or other mark, serving to identify the sample, should be placed on each, and a blank coil, i.e. one containing no milk, should also be rolled up. The coils should then be dried in an oven at 100° C for about one hour.

A sufficient number of Soxhlet extraction flasks should be carefully cleaned, numbered, dried, and weighed. The coils should be placed in the Soxhlet extractors, the corresponding flasks fitted, and a measured volume of dry ether, sufficient to fill the extractor well above the upper portion of the siphon, poured into each. The blank coil should be similarly treated. The extractors are connected to reflux condensers, and the ether boiled by partially immersing the flasks in water at 50° to 60° C. Extraction should be continued for three hours.

The ether should be distilled off, and the flasks placed in a drying oven at 100° C for about 20 minutes, air being carefully blown in every 5 minutes to remove ether vapour. This time is sufficient to dry them, if dry ether has been used. After cooling, in exactly the same way as when the initial weight of the flask was determined, the flask is again weighed. As a check on the correctness of the weight, the flask is then placed in the oven for a further period of 20 minutes, blown out with air, and the weight again taken.

The blank is used to correct for the small amount of " extract " obtained from the paper.

The connections between the flasks and the extractors, and between the extractors and condensers, are preferably made by " all-glass " joints, but they may be made with corks, provided these have been well extracted by ether.

B.D.H. 0·720 ether, or ordinary ether as prepared below, may be used in place of dry ether without affecting the results greatly.

It is advisable to check each batch of strips against the Röse-Gottlieb or some other process, as low results may possibly be obtained if a thick layer of dried milk forms on the paper through the inability of the latter to adsorb readily the amount of milk pipetted on to its surface.

Dry ether is prepared by washing the commercial preparation with water, shaking the washed ether with fused calcium chloride, and distilling, after allowing it to stand over the calcium chloride for a day or two.

Ether sufficiently pure for most purposes may be obtained by distilling (from a water-bath not exceeding 40° C in temperature) the commercial product from a flask to which a fractionating column is fitted. The first fractions, boiling below 34·3° C, and the last, boiling above 34·8° C, should be rejected.

Table 16.1 shows the amount of difference that may be expected between the two modes of procedure.

Table 16.1—Percentage of fat by wet and dry ether

Dry ether, etc. ..	4·49	4·59	0·19	2·61	3·09	3·42	3·05	4·21
Ordinary ether, etc. ..	4·58	4·61	0·28	2·68	3·13	3·45	3·05	4·34
Difference	0·09	0·02	0·09	0·07	0·04	0·03	..	0·13

The average difference is found to be 0·06.

(B) VOLUMETRIC PROCESSES

All the rapid volumetric methods of fat determination depend on the liberation of the fat by the action of sulphuric acid on the milk in specially shaped, calibrated glass containers, which are then centrifuged to aid the separation of the fat, the volume of which is finally read off against the percentage scale etched on the appropriate part of the apparatus.

The Gerber process

This method, originally devised by Dr. Gerber, is to-day used more than any other in this country for the determination of the fat percentage of milk. It is easy to operate and will give results within 0·1 per cent of the values obtained by the Röse-Gottlieb process. As indicated above, the process depends on the action of sulphuric acid on milk, the separation of the fat being aided by the addition of a little amyl alcohol and subsequent centrifuging.

APPARATUS

The following apparatus is needed to carry out the test: an adequate number of Gerber test-bottles with stoppers and a stand to support them; pipettes for measuring milk and amyl alcohol; an automatic measure for sulphuric acid; a water-bath; and finally a Gerber centrifuge. It is proposed to describe the apparatus and chemicals used and then to give details of the method of operation. The process is also suitable for testing cream, cheese, skim-milk, butter-milk, etc., and for convenience, details of the procedure to be adopted in testing these products are also included in this chapter.

Gerber butyrometers. These are glass vessels approximately 195 mm long, and consist of a cylindrical body about 23 mm wide, at one end of which is a neck closed by a rubber stopper and at the other end a graduated glass tube, ending in a small, tapered glass bulb. For milk analysis, butyrometers can be obtained calibrated to read in tenths up to 4, 5, 6, 7, 8, and even 10 per cent of fat. The construction of the graduated tube of the butyrometer is of great importance, both from the points of view of ease in reading the results and of resistance to breakage. We prefer tubes of flat cross-section, graduated to read up to 5 or 6 per cent of fat, and we find that flat tubes which have an external diameter of approximately 11 to 12 mm are very much to be preferred, from the point of view of resistance to breakage, to those of external diameter of only 8 to 9 mm. The basis of calibration used is that a volume in the graduated tube of 0·1 ml should correspond to 0·8 per cent of fat when 11·22 g of milk of average composition are used for the test (Richmond 1905). All butyrometers should be marked with a distinguishing number.

Butyrometer stoppers. These are made of soft rubber and may resemble in shape two ordinary rubber stoppers placed back to back, or may be in the form of a cylinder with rounded ends. They are inserted to half their length into the ringed neck of the butyrometer, and before the reading of the fat is made, their position is adjusted so that the lower edge of the fat coincides with one of the divisions on the scale. Another form of stopper for use in butyrometers with plain necks is the " lock stopper ", which consists of a metal ring into which is fitted a hollow rubber stopper. A key is provided which is inserted into the hollow stopper and by pressure elongates the stopper, which is then readily fitted into the neck of the tube; further manipulation of the key enables the position of the fat column to be easily adjusted.

Butyrometer stands. These are usually made of wood and resemble double test-tube stands fitted with perforated plates, so that the tubes, when placed in the stand for filling, are supported at either end and are maintained in a vertical position. An additional refinement is a removable cover which can be fastened over the stand, thereby enabling a number of tubes to be shaken at once without withdrawing them from the stand. Work is facilitated if the numbered tubes are kept permanently in the stand in a definite order, and it will also be found of assistance, where the necks of the tubes vary in size, if the stopper corresponding to each tube is kept in another stand, which can easily be made by drilling a number of suitable holes in a piece of wood about 1 in. thick.

Milk pipette. This is an 11 ml pipette, calibrated as usual by means of water. The test is usually, therefore, described as carried out on 11 ml of milk. This is not strictly correct, as the average delivery of normal milk at 15·5° C from the type of pipette used has been determined experimentally by Richmond (1905) to be 11·22 g and by Day and Grimes (1918) to be 11·24 g, whereas if a full 11 ml of milk of average specific gravity 1·032 had been delivered, the weight would be 11·35 g. It is therefore more correct to describe the test as made on 11·22 g of milk.

Amyl alcohol pipette. A short form 1 ml pipette is recommended; or alternatively, an automatic measure of the separating-funnel, double-stopcock type, calibrated to deliver 1 ml, may be used.

Automatic measure for sulphuric acid. Where a number of samples have to be examined, it is convenient to use an automatic measure of the type suggested for amyl alcohol, but in this case calibrated to deliver 10 ml. Where only a few samples are used, a 10 ml pipette may be used; in view of the strength of acid used, it is advisable that this pipette should be fitted with one or more guard bulbs.

Water-bath. This should preferably be made of copper and be of sufficient depth to cover the butyrometer tubes when in a vertical position, above the level of the fat column. The bath should be fitted with horizontal perforated plates to hold the tubes vertically, and should also carry a thermometer, so that the temperature of the water can be adjusted to 65° C.

Gerber centrifuge. This type of centrifuge is different from the usual type of laboratory centrifuge in that, instead of the cups falling to a vertical position when the instrument is at rest, they are fixed by means of clips to a solid, almost horizontal disc of metal, the whole rotating about the spindle of the instrument, with the result that the plane in which the cups lie never varies, at whatever speed the instrument is revolving. The disc and tubes are covered by a close-fitting steel cover, which also revolves with the disc, and the whole may be enclosed within a protecting screen. Gerber centrifuges are designed to take from 4 to 24 tubes and may be electrically or hand-driven. They should be capable of a constant speed, when fully loaded, of from 1,000 to 1,200 revolutions per minute.

Gerber sulphuric acid. The acid used should be of 1·820 to 1·825 specific gravity at 15·0° C (containing 90 to 91 per cent of sulphuric acid). The specific gravity may be taken with a hydrometer. Should the temperature not be exactly 15° C (59° F), the specific gravity may be corrected by adding on 0·001 for each degree Centigrade (or 0·00056 for each degree Fahrenheit above 59°) above 15°, or by subtracting 0·001 for each degree below 15°; thus if the temperature be 20° C and the specific gravity 1·818, the corrected specific gravity will be $1{\cdot}818 + (5 \times 0{\cdot}001) = 1{\cdot}823$; and if the temperature be 11° C and the specific gravity 1·827, the corrected specific gravity will be $1{\cdot}827 - (4 \times 0{\cdot}001) = 1{\cdot}823$.

The following mixture is satisfactory—

One thousand eight hundred ml of concentrated sulphuric acid (approximately 98 per cent sulphuric acid) to 200 ml of water, containing 10 ml of 10 per cent ferric chloride or an equivalent amount of ferric sulphate. This mixture possesses the added advantage that it will also indicate the presence of formaldehyde, nitrates or nitrites in the milk; formaldehyde producing a violet coloration on shaking the tube, and nitrates or nitrites producing a golden-brown colour (Elsdon and Sutcliffe, 1913).

Amyl alcohol. Various standards have from time to time been put forward for amyl alcohol intended for use in the Gerber process, but undoubtedly the best criterion of suitability is that the specimen of amyl alcohol, when used in the Gerber process, should give results within 0·05 per cent of those determined by gravimetric methods (Golding 1933). In addition, the amyl alcohol should pass the requirements of the B.S. for amyl alcohol for use in the Gerber test given on page 46 of B.S. No. 696 (Part 2).[1]

O'Sullivan (1935) has shown that amyl ether, either pre-formed in the amyl alcohol or formed during the reaction, can give high results in the Gerber process, and he suggests the following test to detect its presence under the conditions of the Gerber test—

Mix equal quantities of Gerber acid (1·820 to 1·825) and water, cool and adjust to a specific gravity of 1·510 (about 18·6 N). Place 20 ml in a Gerber bottle, add 2 ml of the sample, insert stopper, mix and centrifuge. No oily layer or globules should appear. Invert the bottle in a beaker, keep it lightly stoppered, and heat in water to 80° C for approximately 30 minutes. Centrifuge. No oily layer or globules should appear. Add 2 ml of water, mix, cool and allow the bottle to stand overnight. Centrifuge. No oily layer or globules should appear. (The appearance of an oily layer is always preceded by cloudiness.)

PROCEDURE. The following is a description of the procedure adopted by the revisers in carrying out the Gerber test.

Place a sufficient number of butyrometer tubes in the stand, open end upwards; run 10 ml of the sulphuric acid into each, care being taken not to wet the inside of the neck of the tube. Eleven ml of the mixed sample of milk are allowed to run, gently at first, from the pipette down the sides of the butyrometer tube and to float on the surface of the acid; the pipette is allowed to drain for 4 sec, and then touched on the side of the tube at the lower end of the neck. The pipette should be inserted into the tubes in such a way that the milk does not wet the inside of the neck. One ml of amyl alcohol is then added, taking similar precautions against wetting the neck of the tubes. The stoppers are then firmly inserted and the butyrometers well shaken until the curd is dissolved. The butyrometers are inverted 2 or 3 times, so that the acid in the graduated tubes and in the smaller bulbs may be thoroughly mixed with the bulk of the liquid. The tubes are then placed in the water-bath at 65° C for 5 minutes, and the fat columns adjusted by means of the stoppers to coincide with the scale. The tubes are then rapidly transferred to the centrifuge; they are whirled at a speed of 1,100 revolutions per minute for 4 minutes and then replaced in the water-bath at 65° C for 5 minutes. The percentages of fat are quickly read off by holding the butyrometers up to the light at eye-level, and by slight adjustment of the stopper making the lower level of the fat coincide with one of the larger graduations on the scale; the reading is then taken from this point to the bottom of the upper meniscus of the fat column. This adjustment and measurement should be repeated as a check on the reading. With separated, homogenised and sterilised milks, it is advisable to re-whirl the tubes for a further 5 minutes, as low results are sometimes obtained at first, due to the slowness with which the fat rises in these products. If the fat is not in a clear, limpid layer in the graduated tube, or if the upper layer is frothing, the rotation has not been sufficient and must be repeated, and the speed of the centrifuge should be checked. If the fats are discoloured or otherwise unsatisfactory, the strength of the acid should be investigated.

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[1] B.S. 696 (Part 2)—1936, The British Standards Institution, 28 Victoria Street, London, S.W.1.

It should be noted that the pipettes are graduated to run out; therefore the liquids must not be blown out.

The sulphuric acid should be kept stoppered when not in use.

When loading the centrifuge with tubes it is important that the disc should be evenly balanced; in other words, the tubes should be dispersed symmetrically among the available cups. If this is not possible with the number of samples available, a further tube, which may be a duplicate of one of the samples or may be a tube filled with an equal weight of water, is used to obtain symmetry.

To clean the butyrometers. After reading, place the bottles in the stand, stopper upwards; take out the stoppers and wash them several times with hot water. Do not use soda. Empty the bottles, while the contents are still hot, into a suitable vessel and fill them with *hot* water; empty this out completely and repeat twice; if not quite clean, run a brush down them and wash again. Invert the stand and let the bottles drain. Dry the stoppers after use. Never leave pipettes dirty.

If, in addition to the above, the butyrometers and pipettes are filled with chromic acid solution each week-end and allowed to stand at least overnight, no difficulty should be experienced in keeping the apparatus in good condition.

Calibration of butyrometers. Gerber butyrometers can be obtained bearing the National Physical Laboratory stamp and, if necessary, certificates can be obtained giving the actual errors found, and the use of these tubes is recommended wherever possible. As previously stated, tubes are standardised so that 0·1 ml in the graduated tube corresponds to 0·8 per cent of fat, when 11 ml (11·22 g) of milk of average composition are used for the test. The volume of the graduated part of the tube can therefore be checked by weighing the amount of pure mercury required to fill this part of the tube at room temperature, and correcting the result to ml by dividing by the specific gravity of mercury; this figure is then compared with the graduations on the tube, and any deviation from the above standard noted. The following method of calibration is due to Day and Grimes (1918) and has several advantages—

A small pipette is made by blowing a bulb in the middle of a piece of thick-walled tubing of about 1·5 to 2 mm bore. One end is drawn out to a fine point and a scratch is made above the bulb. The pipette should have a volume of about 0·4 to 0·5 ml, equivalent to about 3·5 scale divisions. The weight of paraffin oil (kerosene) delivered is then determined. This we have found to vary by not more than 1 mg on either side of the mean value. From the sp. gr. of the paraffin, the volume and the value in butyrometer scale divisions can be calculated and marked on the pipette. Paraffin is specially suitable on account of its low viscosity and very slight tendency to evaporate at ordinary temperatures.

The small bulbs of the butyrometers to be calibrated are filled with water up to the graduations, and a pipette-ful of paraffin run into each. They are then centrifuged, small bulb down, and the readings taken to ·01 per cent. A few drops of water are then added to bring the oil layer to the other end of the scale, and the centrifuging and reading are repeated. The two readings will show any serious want of uniformity in the bore of the graduated tube. It is a distinct improvement to take the readings through a piece of tubing about 6 cm long and 3 to 4 mm bore, cut off square and fixed in a clamp. By placing the scale firmly against one end of this tube and reading through the middle of it, parallax is avoided and the mean error of reading reduced from ·01 to ·005. There is a well-marked meniscus at each end of the paraffin layer, so that no correction is required, and both upper and lower readings are obtained with one handling of the butyrometer, instead of two as required in mercury calibration. The butyrometer must be free from grease, or difficulty will be met with in getting a good lower meniscus.

Dolby (1949b) has described a burette for the rapid checking of Gerber butyrometers. It is connected by an S-shaped tube to a long jet which allows mercury to fall gently into the butyrometer without splashing to scatter globules or occlude air bubbles.

Gerber process applied to other dairy products

Separated milk, skim-milk, whey and buttermilk. The determination is carried out in exactly the same way as for milk. It is advisable to use butyrometer tubes reading only up to 4 or 5 per cent of fat, as greater accuracy is thereby obtained. As previously mentioned, separated milks should be re-whirled to obtain the maximum reading. It is advisable to use Siegfeld tubes, which take double quantities of reagents and of separated milk, when dealing with this fluid. These tubes are graduated to $\frac{1}{50}$th per cent, and greater accuracy is thereby obtained.

Condensed milk, sweetened or unsweetened, may be tested by weighing about 20 to 25 g, making up to 100 ml and treating as a milk; longer whirling is, however, necessary to get up all the fat. The percentage of fat found must be multiplied by 100 and divided by the weight taken.

Cream. Special butyrometer tubes may be obtained which give direct readings up to 60 or 70 per cent fat on a standard amount of 5 g of cream. When calibrating these tubes it should be noted that 60 per cent of fat in the tube occupies 3·34 ml. A determination is carried out as follows. Five grams of the well-mixed cream are weighed directly into the butyrometer tube; 10 ml of water and 10 ml of Gerber sulphuric acid are mixed in a small beaker and, while still hot, between 15 and 20 ml of the mixture, depending on the size of the Gerber tube, are added to the cream. One ml of amyl alcohol is finally added and the determination then proceeds as for milk.

Alternatively, ordinary Gerber milk butyrometer tubes may be used for the determination of fat in cream. When the cream contains not more than about 30 per cent of fat it can be measured with great accuracy. In the case of thin cream, i.e. one with not more than 32 per cent of fat, after the acid has been added, add 8·2 ml of water, measure the cream with a 3 ml pipette, filling it up accurately to the mark while in a vertical position, turn the pipette in a nearly horizontal position, and wipe the stem perfectly dry; hold it over the bottle in a vertical position and, removing the finger from the top, let the cream run out freely; after the quick succession of drops has run out, allow three more drops to enter the bottle; add 1 ml of amyl alcohol, and then proceed as in analysing milk.

Calculate the results from Table 16.2, columns 2 and 5 (p. 351).

Table 16.2—For calculating fat in cream by Gerber method

Degrees	Undiluted	Diluted	Degrees	Undiluted	Diluted
8·5	33·2	66·2	6·7	25·9	51·6
8·4	32·8	65·4	6·6	25·5	50·8
8·3	32·4	64·6	6·5	25·1	50·0
8·2	32·0	63·8	6·4	24·7	49·2
8·1	31·6	62·9	6·3	24·3	48·4
8·0	31·2	62·1	6·2	23·9	47·6
7·9	30·7	61·3	6·1	23·5	46·8
7·8	30·3	60·5	6·0	23·1	46·1
7·7	29·9	59·7	5·9	22·7	45·3
7·6	29·5	58·9	5·8	22·3	44·5
7·5	29·1	58·1	5·7	21·9	43·7
7·4	28·8	57·3	5·6	21·5	42·9
7·3	28·3	56·4	5·5	21·1	42·1
7·2	27·9	55·6	5·4	20·7	41·3
7·1	27·5	54·8	5·3	20·3	40·5
7·0	27·1	54·0	5·2	19·9	39·7
6·9	26·7	53·2	5·1	19·5	38·9
6·8	26·3	52·4	5·0	19·1	38·1

Creams containing more than 32 per cent of fat must be diluted. Take two beakers and counterbalance them on a rough balance turning to 0·01 g, pour about 25 g of cream into one and add separated milk or water to the other till the weights are equal, mix the cream and separated milk or water, and measure as before. Use columns 3 and 6 for calculating the results.

This table should be checked by a gravimetric method, and may require a slight correction added or subtracted, which may vary with each pipette.

The cream should be as near 15·5° C (60 °F) as possible, but the error due to temperature is very small and is less than the errors of reading, etc.

The pipette does not deliver the same weight of a cream with 20 per cent of fat as of one with 30 per cent of fat; this has been allowed for in the table.

A convenient and rapid method for cream containing between 22 per cent and 50 per cent fat, where an accuracy of not more than $\pm 0{\cdot}5$ per cent fat is required, is the following. 1·1 g of cream is rapidly weighed out in a small flat porcelain dish and transferred to a butyrometer containing 10 ml of sulphuric acid by means of a jet of warm water from a wash-bottle. One ml of amyl alcohol is added, together with sufficient water to bring the contents of the tube to the usual volume, and the test completed as usual.

To obtain the percentage of fat, the butyrometer reading is multiplied by ten and 1 per cent subtracted from the result, e.g. butyrometer reading 4·35, per cent fat $= 43{\cdot}5 - 1 = 42{\cdot}5$.

Sour milk. Mix the sample well by whisking for a few minutes with a brush made of fine wires; pour about 15 g into a small beaker and weigh;

transfer from 10 to 11 g to the bottle and weigh again to get the weight added; add water to make up 11·22 g. Proceed as before.

$$\text{Calculate: Fat in sour milk} = \text{Reading} \times \frac{11{\cdot}22}{\text{wt. taken}}.$$

An alternative method is to add to each 100 g 5 ml of strong ammonia, shake well, and treat as a milk; increase the result by one-twentieth.

Cheese—The special 5 g butyrometer tubes used for the analysis of cream (*above*) may also be used for the determination of fat in cheese. Five g of thin gratings or shavings of the well-mixed cheese are weighed directly into the tube, and after adding dilute[1] Gerber acid and amyl alcohol as for cream, the contents are mixed and digested, with occasional shaking, in a water-bath maintained at 65° C until the curd is dissolved. This digestion may take an hour or so, depending on the hardness of the sample; the tube is then whirled and the fat read off in the usual way.

Clotted cream, butter, etc. Mix the sample thoroughly (with butter, it is advisable to melt it in a closed vessel at about 40° C (104° F) and to shake violently until solid). Weigh out approximately 2·5 g into a 5 g Gerber cream-tube, add a correspondingly larger amount of dilute[1] Gerber acid, and proceed as for cream. To obtain the percentage of fat in the sample, multiply the fat reading by 5 and divide by the weight taken.

Gerber recommended a modified butyrometer tube with stoppers at both ends for the determination of fat in solid products. Stokes also used a modification of this type of tube, with or without a centrifuge, for the determination of fat in milk. There does not appear to be any real advantage in the use of these modifications, and there is of course the added danger of a stopper becoming loose; the revisers prefer, therefore, to use the ordinary form of Gerber butyrometer.

Specifications for Gerber apparatus and methods

The British Standards Institution have published B.S. No. 696—1936,[2] in two parts (Part I—Apparatus, Part II—Methods), dealing with the determination of the percentage of fat in milk and milk products by the Gerber method. Part I describes in detail butyrometer tubes and ancillary apparatus for the determination of fat in milk, skim-milk, separated milk or buttermilk, cream, and cheese. Part II gives detailed instructions for carrying out the determination with the above apparatus on milk, skim-milk, separated milk and buttermilk, cream, cheese, and dried milk. It also includes a table for determining the percentage of fat in cream, cheese and dried milk by means of the ordinary Gerber milk butyrometers. As previously mentioned, this part includes a British Standard for amyl alcohol for use in the Gerber test. Finally, there is a description of the Röse-Gottlieb method for the gravimetric determination of the fat in milk and a diagram of the apparatus suggested for carrying out the test.

The Leffmann-Beam process

Leffmann and Beam (1892), realising that the time of whirling necessary in Babcock's method, which consisted in treating the milk with an equal volume of strong sulphuric acid and separating the fat by centrifuging, was a disadvantage, experimented with a view to shortening this. They finally decided on the use of amyl alcohol as a means of assisting the fat to rise, and thereby were enabled to reduce the time of whirling.

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[1] Equal volumes of water and Gerber acid.

[2] The British Standards Institution, Publications Dept., 28 Victoria Street, London, S.W.1.

The method has been subjected to a close investigation by Richmond and is of considerable accuracy.

The type of centrifuge used is different from the Gerber centrifuge in that the metal cups take up vertical positions when the machine comes to rest, thereby preventing the spilling of fat from the unstoppered Leffmann-Beam bottles. Hand- or electrically-driven centrifuges are obtainable; they should be operated at a speed of 1,200 revolutions per minute, and power-driven centrifuges, at least, should be protected by a steel screen and cover, which can be securely fastened by means of screw clamps.

The following comparative statement will show the differences of detail between the Gerber and the Leffmann-Beam processes—

Leffmann-Beam	*Gerber*
Test-bottles are flask-shaped.	Test-bottles are butyrometer-shaped.
Ninety-six per cent sulphuric acid is used.	Ninety to 91 per cent sulphuric acid is used.
A mixture of amyl alcohol and hydrochloric acid is employed.	Amyl alcohol alone is employed.
Fat is read off either cold or warm.	Fat is read off at 60° to 70°C.
Bottles are used open.	Bottles are stoppered.

Apparatus. The test-bottles consist of flat-bottomed flasks with a sloping upper portion terminating in a graduated neck. The bottles (English make) hold 29 ml; the necks are made of glass tube 5·96 mm in internal diameter, and are so graduated that 80 divisions = 1·475 ml. These dimensions are according to a specification laid down by Richmond, and differ slightly from those prescribed by Leffmann and Beam. The pipettes used are—

15 ml for milk,
9 ml for sulphuric acid,
3 ml for amyl alcohol mixture,
4·5 ml for cream, and
10·5 ml for water.

Automatic measuring apparatus and burettes may also be used for measuring the acid and amyl alcohol.

Richmond has devised a burette specially for the measurement of sulphuric acid and other corrosive liquids. It has been found in practice that ordinary burettes are liable to be filled to overflowing, and that considerable inconvenience is caused by spilling strong sulphuric acid.

An ordinary burette with a three-way tap is used, and to the tube, for filling from the bottom, a wider tube, ½ inch in diameter and 3 inches long, is fused. An india-rubber stopper is inserted in this, and through it is passed a long glass tube bent as a siphon, which serves to convey the acid from a stock bottle above.

In the top of the burette an india-rubber stopper is fixed, through which passes a tube going almost to the top of an air chamber of glass; from the bottom of the air chamber a glass tube of small bore passes upwards so far as just to enter into the stock bottle.

The illustration (Fig. 3) will make the construction clear.

The conditions necessary for satisfactory working are—

(1) The capacity of the air chan and tube leading to the stock bottle mu not be more than $\frac{1}{5}$ the capacity of the burette.

(2) The bottom of the stock bottle must be well above the top of the tube leading into the air chamber.

The tube leading into the air chamber must be adjusted to the mark on the burette equal to the capacity of the tube.

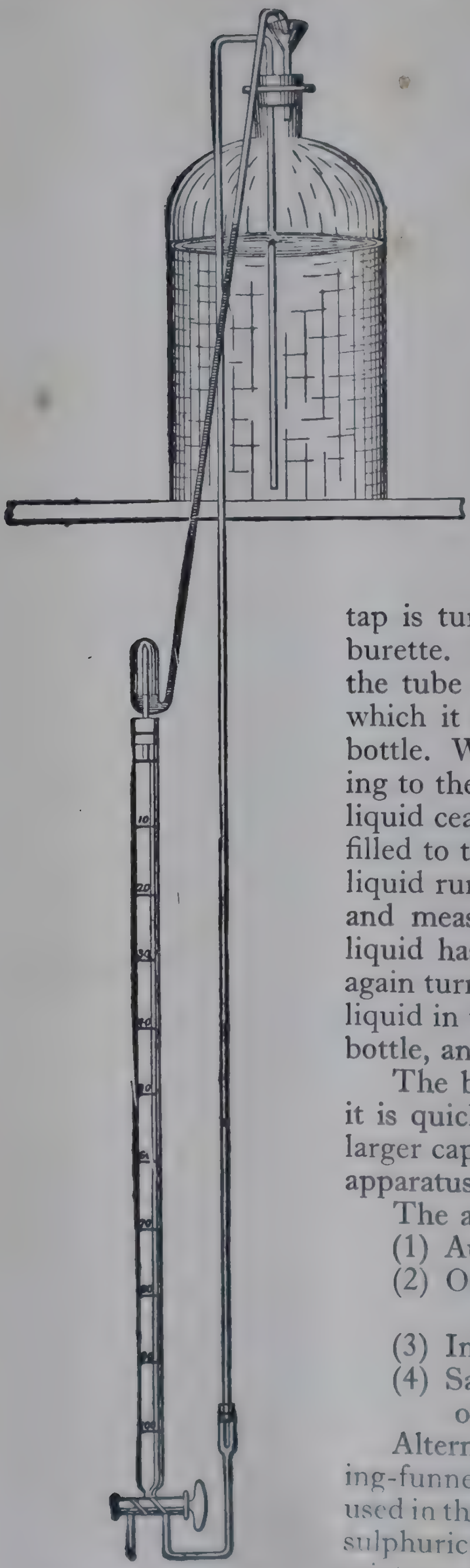

Fig. 3—Automatic burette

The burette is used as follows. The tap is turned so that the liquid enters and fills the burette. As it reaches the upper portion, it passes up the tube and overflows into the air chamber, from which it is forced up the tube leading to the stock bottle. When the liquid reaches a height corresponding to the level of the liquid in the stock bottle, the liquid ceases to run, and the burette is automatically filled to the zero point. When the tap is turned the liquid runs out, air bubbling in from the stock bottle, and measured quantities may be taken. After the liquid has been run out as far as desired, the tap is again turned, and the liquid enters the burette. The liquid in the air chamber is forced back into the stock bottle, and the burette automatically fills itself.

The burette can be made of 9 ml capacity, b it is quicker to employ a graduated burette of mu larger capacity than any form of automatic measuri apparatus.

The advantages claimed for the burette are—

(1) Automatic filling to zero point.
(2) One turn of the tap only required to fill an to measure.
(3) Impossibility of spilling corrosive liquids.
(4) Saving of time, as the filling is done while other operations are conducted.

Alternatively, automatic measures of the separating-funnel, double-stop-cock type, delivery 10 ml, as used in the Gerber process, may be used for measuring sulphuric acid and the amyl alcohol-hydrochloric acid mixture; by running out not quite the whole of the measured 10 ml a sufficient approximation to 9 ml of sulphuric acid is obtained, and by etching two marks on the 10 ml measure, thereby dividing it into thirds, and emptying the measure one-third into each bottle, a sufficient approximation to the 3 ml of the amyl alcohol mixture is obtained.

Reagents. Sulphuric acid containing 96 per cent sulphuric acid should be used for the test, and this has a specific gravity of 1·842 at 15·5° C (60° F). Owing to the fact that strong sulphuric acid has a somewhat anomalous specific gravity, it is not advisable to test the specific gravity directly. The following test will give good results.

Measure accurately 200 ml of acid into a large flask, and to it add cautiously 15 ml of water, cooling the flask by immersion in cold water. Take the specific gravity of this diluted acid, either with an accurate hydrometer or by other means. If the temperature be not exactly 15·5° C, add on 0·001 for each degree Centigrade above 15·5°, or 0·00056 for each degree Fahrenheit above 60° F (or subtract for temperatures below).

The following table will give the strength of acid—

Specific gravity of diluted acid	*Per cent* H_2SO_4
1·8380	98 (94·20)
1·8349	97 (93·22)
1·8311	96 (92·24)
1·8268	95 (91·26)
1·8217	94 (90·28)

The figures in parenthesis give the percentage of sulphuric acid in the diluted acid, the other figures referring to the percentage of acid before dilution.

Use purified amyl alcohol, free from petroleum, specific gravity 0·815 to 0·818 at 15·5° C (60° F), which completely dissolves to a clear liquid when mixed with an equal bulk of hydrochloric acid; this mixture must not become darker than sherry in three days. It is advisable to compare the results given by this process with those given by the Röse-Gottlieb process whenever a new stock of sulphuric acid and particularly of amyl alcohol is used.

The amyl alcohol is mixed with an equal volume of concentrated hydrochloric acid for use; it is best not to keep this mixture longer than a few days.

PROCEDURE

Testing of milk, skim-milk, buttermilk and whey. Measure 15 ml each of the well-mixed samples into test-bottles, holding the point of the pipette against the side of the neck, so that the liquid will run down, and allowing room for the air to escape. Add 3 ml of the mixture of amyl alcohol and hydrochloric acid and shake. Pour in, by small quantities at a time, with frequent shaking in a rotatory manner, 9 ml of sulphuric acid, so that it washes down any particles of milk on the neck of the bottle. When all the acid has been added, the casein will be found to be completely dissolved, and the liquid will have a reddish-brown colour (note, under the Gerber process, the colour produced when formaldehyde, nitrites and nitrates are present). A little practice is required, particularly in warm weather, to mix in all the sulphuric acid without the liquid boiling over, owing to the heat evolved on mixing sulphuric acid with water, but when the correct method is once learned there is no difficulty in doing this. Fill up the bottles nearly to the zero mark with a hot mixture of 1 volume of sulphuric acid to 2 volumes of water, place the bottles in the centrifuge, and whirl for 3 minutes at 1,200 revolutions per minute. Warm the bottles in a water-bath to bring the fat column on the scale, and read off the number of divisions occupied by the fat. As previously mentioned, the scale is divided into 80 divisions which correspond to a total of 8 per cent of fat, so that the number of divisions read off divided by ten gives the percentage of fat in the milk.

The fat column is read off in the same way as in the Gerber process, viz. from the junction of the fat with the acid to the lowest point of the meniscus on the upper surface of the fat.

Skim-milk and buttermilk should be whirled as soon as possible after mixing; in very hot weather, or if the bottles stand very long after the acid has been added, the fat may be of a dark colour.

The difference between the results of the Leffmann-Beam method and those by gravimetric analysis very rarely exceeds 0·1 per cent of fat.

Testing of cream. If the cream contains less than 32 per cent of fat it can be measured direct by the 4·5 ml pipette; if it is thicker than this, it must be diluted with separated milk. Two beakers are counterbalanced on a rough balance turning to 0·01 g; in one of them, about 25 g of cream are placed, and water is run into the other till the weights are equal. The cream and water are mixed together, and the mixture can be measured. If the cream is sour, a few drops of ammonia should be placed in the beaker before the weights of water and cream are adjusted.

The measurement is performed as follows. Fill the pipette with cream by sucking at the top, and close it with the finger; hold the pipette vertically, and allow the cream to run down till the upper surface is on a level with the mark; turn the pipette to a horizontal position and wipe the stem; then return it to the vertical and, holding the point over the neck of a test-bottle, allow the cream to run out freely; after the quick succession of drops has ceased, allow three more drops to run. Add 10·5 ml of water, and proceed as in analysing milk.

Calculate the results from Table 16.3, using columns 3 and 6 for undiluted cream and columns 2 and 5 for diluted cream. This table should be checked by gravimetric analysis whenever a new pipette is used.

Alternatively, approximately 2 g of cream may be weighed into a small beaker and treated with 20 ml of one-to-one sulphuric acid. The beaker is placed on top of a boiling water-bath for about 2 hours, the contents being occasionally stirred by means of a small glass rod; at the end of this period the curd will have dissolved and the contents of the beaker may be transferred to a Leffmann-Beam bottle, rinsing out with 3 ml of amyl alcohol mixture and small portions of hot one-in-three sulphuric acid; care being taken that every particle of fat is transferred to the test-bottle. The bottle is then centrifuged and read off in the usual way. The reading in divisions multiplied by 15·25 (the average weight of milk of normal composition delivered by the 15 ml pipette) and divided by ten times the weight of sample taken, will give the percentage of fat in the cream.

Testing of sour milk. Weigh in a small beaker about 15 g of the sample, which has been previously well mixed by whisking with a brush formed of fine wires; pour as much as possible into a test-bottle and re-weigh the beaker; the difference will give the weight of the milk taken. Add sufficient water to

Table 16.3—For estimating fat in cream

Reading	Diluted	Undiluted	Reading	Diluted	Undiluted
8·5	63·8	32·0	6·7	49·8	25·0
8·4	63·0	31·6	6·6	49·0	24·6
8·3	62·2	31·2	6·5	48·2	24·2
8·2	61·4	30·8	6·4	47·4	23·8
8·1	60·6	30·4	6·3	46·6	23·4
8·0	59·9	30·0	6·2	45·9	23·1
7·9	59·1	29·6	6·1	45·1	22·7
7·8	58·3	29·2	6·0	44·4	22·3
7·7	57·6	28·9	5·9	43·6	21·9
7·6	56·8	28·5	5·8	42·8	21·5
7·5	56·0	28·1	5·7	42·1	21·1
7·4	55·3	27·7	5·6	41·4	20·8
7·3	54·5	27·3	5·5	40·6	20·4
7·2	53·7	26·9	5·4	39·8	20·0
7·1	52·9	26·5	5·3	39·1	19·6
7·0	52·1	26·1	5·2	38·3	19·2
6·9	51·4	25·8	5·1	37·5	18·8
6·8	50·6	25·4	5·0	36·7	18·5

make up to 15·25 g, and proceed as in analysing milk. The reading, multiplied by 15·25 and divided by ten times the weight taken, will give the percentage of fat in the sour milk.

Testing of cheese. Approximately 2 g of the well-mixed and finely-grated cheese may be weighed into a small beaker and treated with 20 ml of one-to-one sulphuric acid, exactly as in the alternative process suggested for cream. It takes somewhat longer for the curd to disappear with samples of cheese than with cream, but otherwise the process is exactly the same.

Testing of clotted cream. Proceed as in the alternative process for cream.

Cleaning of bottles. Empty while hot into a convenient receptacle, and wash twice thoroughly with hot water; if necessary, run a brush down the neck.

Failures and their probable causes. The only failures likely to occur will be evidenced by (1) a dark layer of fat, or (2) a fluffy layer under the fat.

(1) If the acid be too strong, or the temperature too high, or the mixture left too long before whirling, the fat may be dark. The remedy is obvious.

(2) A fluffy layer under the fat is often caused by allowing the milk and acid to stand too long unmixed. It may sometimes be due to poor quality amyl alcohol.

Grit on the bottom of the bottles may cause fracture while in the machine. Fracture may also occur from too sudden a stoppage after the whirling is completed.

Calibration of bottles. The body of the test-bottle up to the lowest graduation should be capable of taking between 29 and 31 ml. The capacity of the neck between the 0 and 80 marks on the scale should be equivalent to 1·475 ml, measured at 15·5° C. The calibration can be carried out sufficiently accurately for ordinary purposes by filling the bottles with water at 15·55° C to the 80 mark, drying the inside of the necks by means of filter-paper, adding 1 ml of water from an accurate 10 ml burette, and then noticing the amount of water required from an accurate 1 ml burette to fill the neck of the bottle to the 0 mark. When large numbers of test-bottles are used and the accuracy of new bottles has to be frequently determined, it is convenient to make a plummet of glass rod, which, when introduced into an accurately calibrated bottle filled with water at 15·5° C to the 80 mark, will raise the level of the water exactly to the 0 mark; this plummet may then be used in a similar manner to check rapidly the accuracy of new bottles. The ultimate check, however, is that the reagents and test-bottles used should give results which are within 0·05 per cent, or at least within 0·1 per cent, of the results obtained by the Röse-Gottlieb process.

The Babcock method

This is very similar to the Leffmann-Beam process; in fact the latter and also the Gerber process are modifications of Babcock's method. The test-bottles used are similar in shape to, but larger than, Leffmann-Beam bottles, and modifications as in the Gerber process are used for different milk products. The chief differences in the method from the two previous processes are: 17·6 ml (18 g) of milk are used; this is treated with an equal volume of approximately 92 per cent sulphuric acid; no amyl alcohol is used; the centrifuge is heated, and finally the test-bottles are whirled three times, with addition of hot water between the whirlings, before a reading is taken.

When adding the 17·5 ml of sulphuric acid (sp. gr. at 15·5° C, 1·82 to 1·84), care must be taken (as in the Leffmann-Beam process) to add it a little at a time, with constant shaking. The bottles are then immediately placed in a centrifuge heated to about 60° C and whirled at approximately 900 revolutions per minute (the speed depending on the effective diameter of the centrifuge) for 5 minutes; they are then filled with boiling water to the bottom of the neck and whirled for a further 2 minutes. Finally, boiling water is added to bring the fat layer near the top of the graduations, and a third whirling for two minutes is given. The bottles are then placed in a water-bath maintained at approximately 60° C and the fat read off. It is interesting to note that all references to this process state that the readings should be made from the bottom to the very top of the fat column, i.e. to the top of the upper fat meniscus; whereas in the Gerber and Leffmann-Beam processes the readings are made as usual, only to the lowest point of the upper fat meniscus.

This process is not used in Britain to the same extent as the other two processes. It is, however, popular in the U.S.A. and it is official in *Methods of Analysis* (A.O.A.C. 1950), detailed information with regard to the apparatus and mode of carrying out the test being given.

A British Standard, No. 755—1937, published in two parts, describes the method even more fully. Part 1, Apparatus, gives details of test-bottles and other apparatus for the determination of the fat in milk, cream, skim-milk and cheese. Part II, Methods, describes in detail the methods of carrying out the test on the above dairy products. These publications should be consulted by all workers who use this process.

Calibration of test-bottles. As previously mentioned, the test is carried out on 18 g of milk (this being the average weight of milk delivered by a 17·6 ml pipette). For this quantity of milk, 0·2 ml in the graduated part of the neck of the bottle should correspond to 1 per cent of fat. Tested bottles can be obtained bearing the stamp of the National Physical Laboratory, and wherever possible these should be used. Bottles may also be calibrated in the laboratory by the methods used for Leffmann-Beam or Gerber bottles; in this connection, Smit (1923) describes in detail five methods of calibration, viz. (*a*) by burette, (*b*) the plunger method, (*c*) testing with mercury, (*d*) weighing the mercury, and (*e*) the glass rod method; and he tabulates the errors determined by each method.

Herreid *et al.* (1950) have suggested a modification of the Babcock test involving a slight increase in the volume of milk, and elimination of the meniscus on the fat column. The opinion has been expressed that this modification is an improvement on the present standard American method.

General comparison of methods

Workers in the Netherlands have recently studied the accuracy of various methods for determining the fat in milk and milk products. Brouwer (1948) has published corrections for the Gerber method when applied to milk powder. He has given the following equations—

Product	*Equation*
Milk powder	$\text{Fat} = 0{\cdot}868\ V + 0{\cdot}01$
Cheese	$\text{Fat} = 0{\cdot}873\ V + 0{\cdot}085$
Milk	$\text{Fat} = 0{\cdot}872\ V + 0{\cdot}003$
Butter fat	$\text{Fat} = 0{\cdot}876\ V + 0{\cdot}003$

V = volume in ml of fat in capillary or butyrometer

Brouwer concludes that in the ordinary Gerber method there is a loss of 3 mg in testing milk and a loss of 10 mg in testing milk powder.

Radema and Mulder (1948) have studied the Röse-Gottlieb method and expressed the opinion that the Röhrig is less suitable than the Mojonnier tube. They feel that it is essential that the milk should be weighed in the tube and that the milk should be shaken three times with ether.

The phospholipids and sterols are included in this way with the fat.

In their paper on the Gerber method they stress that the Gerber method is empirical and is not quite accurate, using the Röse-Gottlieb method as a standard. They have suggested the following correction formula—

$$\text{Gerber value} = (1{\cdot}04 \times \text{Röse-Gottlieb value}) - 0{\cdot}07$$

One method of applying this correction in practice is to use a pipette delivering 10·77 instead of 11 ml of milk.

While the findings of Mulder and associates may be perfectly true it should be borne in mind that in the United Kingdom, at any rate, the Gerber test is only used for routine control purposes in the dairy industry and as a preliminary screening test by public analysts. No reputable laboratory would rely upon the Gerber test for an accurate determination of the fat content of milk or of any other dairy product. Thus, if a public analyst finds that a Gerber test gives a value which is just at, or just below the legal presumptive limit of 3·0 per cent fat in milk, he invariably follows up with a Röse-Gottlieb or other accurate gravimetric method which is made in duplicate if prosecution is a possibility.

Summing up, therefore, it may be said that while Mulder's results are of great value and interest theoretically it hardly seems worth while to attempt to apply any correction to Gerber results in routine testing.

(C) INDIRECT METHODS

Calculation from specific gravity and total solids

The calculation of total solids from determinations of specific gravity and of fat by means of various formulae, tables, or by the " milk scale ", has been fully described in Chapter 6. By determining specific gravity and total solids, the same methods can also be used to calculate the percentage of fat; this procedure is not often adopted, because of the time involved in determining total solids as compared with the volumetric determination of the fat content.

Calculation from refractive index of an ethereal solution of the fat

This method was recommended by Wollny (1900); it depends on the preparation of an ethereal solution of the fat from a definite quantity of milk, which is first agitated with a standard alkaline copper solution, then with a definite quantity of ether, and finally centrifuged to obtain a clear ethereal layer. A few drops of the latter are then transferred to a Wollny milk-fat refractometer or an Abbé refractometer, the reading determined, and the amount of fat obtained by consulting tables. Details of the method, together with the necessary tables, are given by Leach (1920). Although this method has had considerable use on the Continent it has not been adopted in this country, and it will obviously tend to give high results, due to the evaporation of ether from the solution during its transference to the refractometer.

An interesting micro-method for the determination of fat in skim milk and non-fat dry milk solids has been devised by Heinemann and Rohr (1950). The method consists essentially in measuring the monolayer of the fatty extract formed by transferring about 0·0057 ml of an ether solution to a prepared surface on a solution of 0·2 per cent acetic acid.

The list of methods for the determination of milk fat described in this chapter is not by any means exhaustive, as a search through the literature will quickly prove. Most of the processes used by workers in Britain have, however, been included under the headings of gravimetric and volumetric methods.

CHAPTER 17

DETERMINATION OF PROTEINS, SUGARS, CITRIC ACID AND LECITHIN IN MILK

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(1) THE DETERMINATION OF PROTEINS

Proteins may be determined collectively as total proteins, or separate determinations of casein and albumin can be carried out.

Kjeldahl's method for the determination of total nitrogen

This method is the most convenient for the routine determination of total nitrogen in milk and is carried out as follows. Ten millilitres of the sample are pipetted into a Kjeldahl digestion flask and the weight is arrived at by using the same pipette and delivering at the same rate as when weighing out the quantity for total solids. Ten grams of potassium sulphate, 25 ml of concentrated sulphuric acid and about 0·1 g of selenium or a crystal of copper sulphate are added and the whole boiled—care being taken that the flame does not come above the level of the liquid—until colourless, and then for a further 2 hours. The flask is allowed to cool and the contents are carefully diluted with water and transferred to a distillation flask of about 1,000 ml capacity. The bulk of liquid in the distillation flask should fill about half the capacity of the flask. Add a few drops of litmus solution and about 5 g of granulated zinc (to prevent bumping) and finally about 75 to 80 ml of 50 per cent by volume sodium hydroxide solution, which should be carefully poured down the neck of the flask to form a layer under the acid liquor. The flask is then attached by means of an adapter, fitted with a spray trap, to a distillation apparatus, the receiver of which contains 50 ml 0·1 N hydrochloric acid or sulphuric acid and a few drops of methyl red indicator solution. The contents of the distillation flask are now carefully mixed and should be alkaline, as indicated by the litmus solution. The solution is now distilled, and the whole of the ammonia should pass over in about 250 ml of distillate; this can be ascertained by testing the drops from the condenser with litmus paper. Finally, the excess acid remaining in the receiver is back-titrated with 0·1 N sodium hydroxide to methyl red indicator.

A blank experiment, carried out exactly as in an actual determination except that no milk is used, is made on each batch of reagents, and this is usually found to be equivalent to about 0·3 ml 0·1 N hydrochloric acid.

The amount of 0·1 N acid neutralised by the ammonia from the sample

$$= (50 - \text{back-titration}) - \text{blank} = x \text{ ml}$$

Then

$$\text{percentage of N} = \frac{x \times \cdot 0014}{\text{wt. of sample}} \times 100,$$

and

$$\text{percentage of protein} = \text{percentage of N} \times 6{\cdot}38.$$

N*

Both casein and albumin, which form by far the greater proportion of the nitrogenous compounds in milk, contain about 15·67 per cent of nitrogen, and therefore the total proteins may be found sufficiently accurately by multiplying the percentage of nitrogen by 6·38. This factor has been generally accepted for many years, and as it is most useful for purposes of comparison it will be used throughout this book, although Richmond (1908) considered that it is slightly low and that 6·39 or 6·40 would be better figures.

An error, due to the inclusion of the non-protein nitrogen, is introduced into this process, but as this is only slight it does not affect the comparative value of the results.

Determination of the aldehyde figure

This method was originally due to Steinegger (1905) and serves as an indirect method of determining the proteins. It may be combined with the acidity estimation, and it depends on the fact that when an amino-acid which has been neutralised is treated with an excess of formaldehyde it becomes acid and requires the addition of a further quantity of alkali to neutralise it.

Richmond's modification of the process is as follows. Ten or 11 ml of milk are neutralised to phenolphthalein with N/11 strontia solution; 2 ml of 40 per cent formaldehyde solution are then added, and the mixture again titrated to the same shade of pink as in the first titration. The acidity developed by the addition of the formaldehyde, less that due to the 2 ml of formaldehyde (determined separately), calculated as degrees, gives the aldehyde figure. In normal milks it varies from 18·1° to 22·6° with an average of 19·9°. The aldehyde figure determined in this way (i.e. the number of ml of N strontia to neutralise 1,000 ml of milk), multiplied by 0·17, gives the percentage of proteins. This is not an absolutely correct measure of the proteins, as casein and albumin do not give the same aldehyde figure, and the relative proportions of these in different samples of milk are liable to slight variation.

Steinnegger originally suggested the use of 0·25 N caustic soda solution for titration purposes, and De Graaf and Schaap (1903) also advocated the use of this solution and gave the factor to convert degrees to percentage of protein as 0·0777 (this equals, when multiplied by 2·5 to bring it to the equivalent of ml N solution per litre, 0·1942, against Richmond's figure of 0·17).

Pyne (1932) has suggested the addition of potassium oxalate to the sample before carrying out the titrations, in order to eliminate the disturbing effects of the soluble calcium salts in the milk.

Total protein from acidity and pH determination

Harris (1925) has shown that a protein in solution can be determined by measuring the amount of standard acid or alkali required to alter the pH value of a definite volume of sample from one value to another, an allowance being made for the acid or alkali required in a protein-free blank to bring about the same pH alteration. The corrected volume of acid or alkali used is proportional to the amount of protein present. The chief constituents of milk which affect the pH value on the addition of acid are casein, lactalbumin, phosphates and citrates; in normal milk, ash and protein are related and so are casein and lactalbumin, with the result that the buffering effect should be directly proportional to the amount of protein. For milk the determination should be carried out between the pH values of 6·7 to 4, and if 10 ml of milk are used the number of ml of 0·1 N HCl required to alter the pH from 6·65 to 5·2 is numerically equal to the percentage of protein in the sample.

Determination of casein

This determination should be made while the milk is fresh. If it is not practicable to make the determination within 24 hours, the American A.O.A.C. recommends the addition of 1 part of formaldehyde to 2,500 parts of milk and keeping in a cool place.

Moir (1931) carried out a very extensive investigation into the best conditions for the determination of casein and other proteins in milk. He concluded that casein was best precipitated by acetic acid and sodium acetate solutions at a pH of 4·6 (the isoelectric point of casein). The following is a description of Moir's method for casein (the original papers should however be consulted by all workers in this field).

Ten millilitres of milk are weighed into a covered beaker of 100 to 150 ml capacity. Dilute the milk with approximately 50 ml of distilled water which has been previously warmed to 40° to 42° C. Add at once 1·5 ml of 1·67 N (10 per cent) acetic acid and then stir *gently* by rotating the stirring-rod four times in the beaker. After allowing the beaker to stand about twenty minutes, add 4·5 ml of 0·25 N sodium acetate solution and, after stirring *gently*, leave for at least an hour. Filter through a 9 cm No. 42 Whatman filter, which is fluted to facilitate filtration. Wash the precipitate with distilled water three times by decantation, followed by two further washings in which the precipitate is broken up and transferred to the paper. Finally, rinse the rim of the filter-paper with a fine stream of water. The filtration process should be carried out without interruption, and the subsequent transference to the Kjeldahl flask of any casein adhering to the beaker and stirring-rod should be carried out before the casein dries. To do this, about 20 ml of water are placed in the beaker and about 5 to 7 ml of concentrated sulphuric acid are carefully poured down the side. The heat generated by gentle mixing helps to dissolve the casein and the solution is then poured into the Kjeldahl flask; this treatment is repeated twice. The filter-paper and the bulk of the casein are then added, followed by the usual amount of potassium sulphate and a crystal of copper sulphate. The water is evaporated over a small flame until the danger of frothing is over, and then the residue is digested until clear. It has been noted that, during digestions, some of the acids from the fat condense in the neck of the flask and may be the cause of subsequent frothing during the distillation. These can be destroyed if the flask is allowed to cool when digestion is nearly complete, and about 50 ml of water are carefully added and mixed with the contents. The fatty material is washed down by the condensation of steam during the evaporation of the water. The determination is completed in the usual way and the ammonia collected in 40 ml of 0·1 N sulphuric acid to which a few drops of sodium alizarine-sulphonate (1 per cent) indicator have been added. The excess of acid is measured by titration with 0·1 N sodium hydroxide solution until the brown colour first develops a pink shade. The result is corrected for a blank determined on the same quantities of all reagents, including filter-paper, and is expressed as per cent of casein nitrogen.

Official A.O.A.C. methods for casein

METHOD I. Place 10 g of the sample in a beaker with 90 ml of water at 40° to 45° C and add at once 1·5 ml of dilute acetic acid (1 + 9). Stir, and allow to stand 3 to 5 minutes. Decant on to an acid-washed filter, wash by decantation 2 or 3 times with cold water, and transfer the precipitate to the filter. Wash once or twice in the filter. The filtrate should be clear, or very nearly so. If the first portions of the filtrate are not clear, repeat the filtration, after which complete the washing of the precipitate. Determine nitrogen in the washed precipitate and filter-paper as usual, and multiply by 6·38 to obtain the equivalent of casein.

To a sample of milk that has been preserved, the acetic acid should be added in small portions, a few drops at a time, with stirring, and the addition should be continued until the liquid above the precipitate becomes clear, or very nearly so.

METHOD II (3rd edn.). To 10 g of the sample add 50 ml of water at 40° C, then 2 ml of potassium-alum solution saturated at 40° C or higher. Allow the precipitate to settle, transfer to a filter, and wash with cold water. Determine nitrogen on the precipitate and filter-paper as usual.

Waterman (1927) suggested the precipitation of casein at its isoelectric point, and the method of Moir described above is based upon this ideal. Waterman also avoids the tedious washing of the precipitate by the determination of the total nitrogen in the milk and in the serum after the precipitation of the casein. This method (A.O.A.C. 1950) is as follows—

Reagent. Pipette 250 ml of N acetic acid into a 1,000 ml flask. Add 125 ml of N CO_2-free sodium hydroxide. Make up to 1,000 ml with CO_2-free distilled water and mix thoroughly.

Determination. Pipette 20 ml of the sample into a 100 ml flask. Add 50 ml of the reagent, mix, make up to volume with distilled water and shake well. Set the flask in water at 50° to 60° C and leave for 15 minutes. Cool to room temperature and filter. Use a double folded paper, returning the filtrate once or twice to the filter; then filter once through a hardened filter-paper. Determine nitrogen (A) in 50 ml of the clear filtrate, and determine total nitrogen (B) in 10 ml of the milk. Then $6{\cdot}38 \times (B - A)$ = casein in 10 ml of milk. Report grams of casein per 100 ml of milk, or divide the grams per 100 ml by the density of the milk and report as percentage by weight.

Volumetric methods for the determination of casein have been suggested by several workers, and a method of this type due to Van Slyke and Bosworth (1909) is as follows. Twenty ml of milk are run into a 200-ml graduated flask, phenolphthalein is added, and then 0·1 N sodium hydroxide solution in small quantities at a time, with constant and vigorous shaking, until a faint but distinct pink tint is permanent. Excess is to be avoided. The solution, which should be at a temperature of from 18° to 24° C, is then treated with 0·1 N acetic acid in quantities of about 5 ml at a time up to 25 ml, and thereafter in quantities of 1 ml until, on standing, the casein separates promptly in large white flakes. The amount of acid added is noted as A. The liquid is then diluted to the mark and the whole vigorously shaken. It is then filtered, when, if the right amount of acid has been added, the filtrate will be practically clear and the rate of filtration will be reasonably quick. One hundred ml of the filtrate are then titrated with 0·1 N sodium hydroxide until a faint but permanent pink tint is obtained. The quantity of hydroxide added is noted as B. Then

$$\text{Percentage of casein} = \left(\frac{A}{2} - B\right) \times 1{\cdot}0964.$$

The process is not satisfactory with milks which are sufficiently sour to curdle on boiling. When the determination cannot be carried out at once, the milk may be kept for about one week on the addition of 1 part in 1,000 of mercuric chloride.

Determination of albumin and globulin together

In view of the low results obtained by simple heat coagulation on the filtrate after the removal of casein, Moir (1931) has suggested the following tentative method for the determination of albumin and globulin. The filtrate from the precipitation of casein by Moir's method (*see above*) is treated with sufficient trichloracetic acid to make the final concentration approximately 4 per cent. It is then heated for 30 minutes on a boiling water-bath, allowed to cool, filtered, and washed with a 1 per cent solution of trichloracetic acid. The nitrogen content of the precipitate is determined by Kjeldahl's method.

Determination of albumin and globulin separately

In view of the small amount of globulin present in milk, the determination of this substance is fortunately not often required. The separate determination of albumin and globulin has attracted the attention of numerous workers, and the difficulty of the problem is indicated by the fact that no method of proved accuracy has yet been evolved. Moir (1931) suggests the following two methods, the first of which is a direct method, for the determination of globulin.

The filtrate from the casein determination (Moir's method) is neutralised to phenolphthalein with 0·1 N sodium hydroxide. It is then saturated with either magnesium or sodium sulphate (anhydrous), the amounts being found approximately by calculation from solubility tables; the salt must be stirred in while the temperature is kept at about 20° C by means of a water-bath. Allow to stand overnight and then complete the filtration and washing (with saturated salt solution) through a pleated filter without interruption. Due to the mechanical impurities often present in the large amount of salt used, it is desirable after washing is complete to wash the globulin through the filter-paper into the Kjeldahl flask, using distilled water or a very dilute solution of sodium chloride.

Alternatively, casein and globulin can be determined together as follows, and the difference after subtracting the casein, determined separately, is reported as globulin.

Ten ml of milk (weighed) are neutralised and mixed with at least 90 ml of saturated magnesium or sodium sulphate solution and sufficient of the solid salt to saturate the 10 ml of sample. The precipitate is filtered, washed with saturated salt solution, and its nitrogen content determined.

Albumin may be determined in the filtrate from either of the above methods by acidifying with 3 ml of 10 per cent acetic acid and heating on the boiling water-bath for at least 30 minutes. Allow to cool, filter, and wash with saturated salt solution. A little soda can be used to facilitate transference to the

Kjeldahl flask of any traces of the precipitate which adhere to the precipitation beaker.

Official A.O.A.C. method for albumin

Exactly neutralise the filtrate obtained from the official method (I) (*see above*) for the determination of casein with 10 per cent sodium hydroxide solution, add 0·3 ml of dilute acetic acid (1 + 9), and heat on a steam-bath until the albumin is completely precipitated. Collect the precipitate on a filter, wash with cold water, determine the nitrogen as usual, and multiply by 6·38 to obtain the equivalent of albumin.

Alternatively, to the filtrate obtained from the casein by the official method (II) add 0·3 ml of dilute acetic acid (1 + 9), boil until the albumin is completely precipitated, and proceed as in the official method for albumin.

An ingenious method for the determination of albumin is suggested by Claudius (1913). The albumin is precipitated by a 2 per cent aqueous solution of trichloracetic acid to which are added 0·5 per cent of tannic acid and 0·1 per cent of acid magenta. The filtrate is then compared in colour with that of the original reagent, the diminution in intensity being a measure of the quantity of albumin precipitated.

Rowland's method for the determination of the nitrogen distribution in milk (Rowland 1938)

(*1*) *Total nitrogen*

Into a weighed 100 ml graduated flask, pipette 5 ml of the milk, weigh and dilute to the mark with water. Pipette 20 ml of the diluted milk (= 1 ml of the original) into a 200-ml Kjeldahl flask, and add 5 ml of nitrogen-free sulphuric acid, 2 g of potassium sulphate, 0·2 g (approx.) of copper sulphate and 2 drops of selenium oxychloride. Digest the contents of the flask by heating over a small flame, continuing the heating for 15 minutes after the contents become clear. Allow to cool, add 50–60 ml of water and excess (15 ml) of sodium hydroxide solution (50 per cent). By steam distillation liberate the ammonia direct from the Kjeldahl flask, through a small bulb trap and condenser, into excess—normally 25 ml—of 0·02 N sulphuric acid to which 2–3 drops of 0·1 per cent methyl red solution have been added. The ammonia is completely removed in 10–15 minutes. Boil the distillate to remove carbon dioxide, cool, and titrate the excess of acid with carbonate-free 0·02 N sodium hydroxide from a 10 ml burette reading to 0·02 ml.

(*2*) *Non-casein nitrogen*

Into a 100-ml graduated flask pipette and weigh 10 ml of the milk. Add 70–80 ml of water at 40° C and 1·0 ml of 10 per cent acetic acid solution. Mix, and after 10 minutes add 1·0 ml of N sodium acetate solution and mix again. Allow the contents of the flask to cool, and make up to the 100 ml mark with water, mix, and stand for the precipitate of casein and fat to settle. Filter on a dry pleated No. 40, 11 cm Whatman paper into a dry flask. Pipette 20 ml of the filtrate into a 200-ml Kjeldahl flask, and continue the digestion and distillation as in (1) using 15 ml of 0·02 N acid to receive the ammonia.

The non-casein nitrogen content of the milk found in this way has to be reduced slightly to correct for the volume occupied in the 100-ml flask by the precipitate of casein and fat. Rowland has determined this factor experimentally and quotes a factor of 0·995 as being satisfactory for general use.

(3) *Non-protein nitrogen*

Into a 50-ml graduated flask pipette and weigh 10 ml of the milk. Dilute to the mark with 15 per cent trichloroacetic acid solution and mix immediately. When the precipitate has settled, leaving a clear supernatant liquid, filter on a dry pleated No. 40, 9 cm Whatman paper into a dry flask. Pipette 20 ml of the filtrate into a 200-ml Kjeldahl flask, and continue the digestion and distillation as in (1) using 10 ml of 0·02 N acid to receive the ammonia.

(4) *Proteose-peptone plus non-protein nitrogen*

The milk may be heated at 95° C for 10–20 minutes and the denatured albumin and globulin precipitated at the same time as the casein by method (2) above. The filtrate then contains only proteose–protein and non-protein nitrogen, and there is no correction for incomplete coagulation. Digest 20 ml of this filtrate and distil into 10 ml of 0·02 N acid.

(5) *Globulin nitrogen*

Pipette 20 ml of the casein-free filtrate from (2) into a 50 ml beaker, add a few drops of bromcresol purple or bromthymol blue solution as an indicator and 0·1 N sodium hydroxide solution until the colour denotes a pH within the range of 6·8 to 7·2. For normal milk samples 2·0–2·5 ml are required. Add powdered crystalline magnesium sulphate, 9 g per 10 ml of solution, and stir until the solution is saturated. Warming to 25–30° C markedly assists solution. Set the beaker aside at room temperature for several hours, or preferably, overnight, and then filter on a small (about 5 cm) No. 40 Whatman paper that has been very finely pleated to assist filtration. Transfer the precipitate to the paper and wash with saturated magnesium sulphate solution. Transfer the precipitate and paper to a 200 ml Kjeldahl flask, and digest as in (1) except that owing to the presence of some sulphate in the water, only about 1g of potassium sulphate should be added. Distil the ammonia into 10 ml of 0·02 N acid.

Calculation of nitrogen in the various protein fractions

Thus the following nitrogen percentages have been determined—

(1) Total N, i.e. casein + albumin + globulin + proteose–peptone + non-protein N
(2) Non-casein N, i.e. albumin + globulin + proteose–peptone + non-protein N
(3) Non-protein N
(4) Proteose–peptone + non-protein N
(5) Globulin N

Thus the total, globulin, and non-protein N are given—(1), (5), and (3)—and the casein, albumin, and proteose–protein N are found by difference, thus—

Casein N = 1 − 2
Albumin + globulin N = 2 − 4
Albumin N = 2 − (4 + 5)
Proteose–peptone N = 4 − 3.

The procedure is much simplified if it is considered adequate for many purposes to include the proteose–peptone N with the albumin N—as has been done in the past when the presence of proteose–peptone substance was not allowed for—to give a single "albumin" figure. In this case determinations (1), (2), (3), and (5) only are required, providing total globulin and non-protein N directly and, by difference, casein N = 1 — 2 and "albumin" N = 2 — 3 — 5. Total protein nitrogen is given by 1 — 3, and soluble protein N by 2 — 3.

The various protein N fractions may be converted into the corresponding amounts of protein by multiplying by 6·38.

Rapid colorimetric method for proteins

The following method, due to Gorffon (1938), enables a protein estimation to be carried out in 20 to 25 minutes. It is based on the colour reaction between Folin and Ciocalteu's reagent and the tyrosine and tryptophan present in milk proteins.

To 1 ml of the milk are added 4 ml of alcoholic sodium hydroxide (0·5 g dissolved in 100 ml of alcohol at 60° C). After thorough mixing 5 ml of ether are added and the whole shaken until the opalescence due to the milk has vanished. Water is then added drop by drop, while the mixture is shaken, until the aqueous layer amounts to 10 ml. 1·5 ml of this are diluted with 25 ml water, and 1 ml of the alcoholic sodium hydroxide added, this being followed immediately by 3 ml of Folin and Ciocalteu's reagent. The whole is made up to 50 ml with water and the intensity of the blue colour measured on a colorimeter or photo-colorimeter (using a red filter) after 5 or 10 minutes. The colorimeter is standardised by resorcinol solution treated in the same manner; a 0·048 per cent solution of resorcinol yields the same intensity of colour as a 1 per cent solution of milk proteins.

Estimation of curd

This estimation is not usually carried out by the analyst but may be of value to the cheese-maker. The following method, due to Richmond, is a modification of a method proposed by Lindet.

Determine the specific gravity and fat in the milk by any convenient method; to 100 ml of milk add 0·01 g of rennet powder, and keep at 42° C till curdled; cut up the curd, allow it to settle, and strain off the whey through muslin; cool the whey to 15·5° C, and estimate the specific gravity and fat as before.

Add the degrees of gravity and the percentage of fat in the milk, and subtract the sum of the degrees of gravity and the percentage of fat in the whey; the difference divided by 3·5 will give the percentage of dry curd available for cheese-making. This will, of course, be very much less than the pressed curd actually obtained, as this not only contains a considerable percentage of water, but also the bulk of the fat in the milk. Roughly speaking, the dry curd multiplied by 4 plus the difference in the percentage of fat in the milk and in the whey will give the curd actually obtained.

(2) DETERMINATION OF LACTOSE AND OTHER SUGARS IN MILK

Lactose, or milk-sugar, is generally determined by difference in routine milk analysis. This is primarily due to the doubt which exists as to the exact amount of combined water which is present with lactose in milk solids as usually determined, and therefore the doubt as to the factor for lactose to be employed in interpreting the figures obtained by direct analytical methods. As hydrated lactose, $C_{12}H_{22}O_{11}$, H_2O, is not completely stable at 100° C, it is possible that milk solids, as ordinarily determined, contain a proportion of lactose in the anhydrous condition. Nevertheless it is often desirable, particularly in examining abnormal milks and milk products, to determine directly the amount of lactose and also to satisfy oneself as to the presence, or otherwise, of other sugars.

A number of processes are available for the determination of sugars, and these may be grouped as follows—

(*a*) Polarimetric methods.
(*b*) Gravimetric methods involving the use of copper solutions.
(*c*) Volumetric methods involving the use of copper solutions.
(*d*) Iodometric and other methods.

Qualitative tests for the presence of sucrose in milk are described on p. 413.

(A) POLARIMETRIC METHODS

The polarimeter is the quickest and probably the most accurate method of determining sugars. With milk it is necessary, before a solution of the sample can be polarised, to remove completely the fat and the proteins, which would otherwise interfere by making the solution too opaque or by polarising to the left. The removal of fat and protein is carried out by precipitation, and the bulk of the precipitate must be allowed for in calculating the concentration of sugar in the remaining solution; the determination of the correct allowance to be made is the chief source of difficulty and error in the examination of milk and milk products.

The rotation of a substance in solution is affected by the temperature, the type of light, and the concentration of the solution. For convenience, therefore, it is usually determined in a solution of approximately 10 per cent concentration at 20° C and by the use of monochromatic light. The specific rotating power under the above conditions may be expressed for sodium light as—

$$\left[a\right]_{D}^{20} = \frac{100\,a}{l\,.\,c}$$

where a is the observed rotation in angular degrees, l is the length of the tube in decimetres and therefore of the column of sugar solution, and c is the concentration in grams per 100 ml of solution.

Pure solutions of simple sugars under the above conditions give the following figures for their specific rotation—

Sucrose	+ 66·5
Dextrose	+ 52·7
Laevulose	− 93·8
Invert sugar	− 20·0
Lactose	+ 55·3
Hydrated lactose, $C_{12}H_{22}O_{11}, H_2O$	+ 52·5
Maltose	+ 139·5

The above figures can be used to determine the concentration of a single known sugar in an otherwise optically inactive solution. Sucrose and another known sugar in solution can also be determined by the Clerget method, which depends on determining the rotating power of the solution, both before and after the cane sugar has been inverted.

In addition to polarimeter scales marked in angular degrees, the instruments known as " saccharimeters " are fitted with an arbitrary sugar scale which is graduated in such a way that when the " normal weight " of pure sucrose is dissolved in 100 ml of solution and observed in a 200 mm tube a reading of exactly 100 divisions is obtained. Divisions, therefore, in these scales correspond directly to percentages of sucrose. The " normal weight " varies with different makes of saccharimeter.

Vieth method

The reagent, " acid mercuric nitrate ", is prepared by dissolving mercury in twice its weight of nitric acid of specific gravity 1·42 and, after solution, adding an equal volume of water; alternatively, 5 ml of mercury are dissolved in 96 ml of strong nitric acid and diluted with an equal volume of water.

Vieth found the volume of precipitated proteins from 100 ml of milk to amount on the average to 3 ml and, consequently, added 3 ml of acid mercuric nitrate solution to allow for this. The method is carried out as follows.

Measure 50 ml of milk into a small flask, add 1·5 ml of acid mercuric nitrate, and mix well by shaking violently; pour the mixture on to a filter, and fill a polarimeter tube with the filtrate; polarise, and correct the reading for that obtained in a blank reading, i.e. by reading a tube filled with water.

As the $[\alpha]_D$ of hydrated milk-sugar is 52·5°, the reading, if in angular degrees, can be converted into percentage of milk-sugar by the formula

$$m = \frac{100 \times 100}{52{\cdot}5 \times l} \times r,$$

where m = number of grams of milk-sugar per 100 ml of solution polarised,
l = length of tube in millimetres, and
r = reading in angular degrees.

If the length of the tube be 200 millimetres, the formula is

$$m = \frac{r}{1{\cdot}05}.$$

The resulting figure, representing milk-sugar in the solution polarised, must be submitted to correction.

The volume of the liquid from which the fat and protein have been precipitated is the volume of the milk *plus* that of the mercuric nitrate *minus* that of the protein precipitate and fat.

As the volume of the mercuric nitrate was made purposely equal to that of the protein, both of these may be neglected, one compensating for the other.

Taking the volume of the milk as 100 ml, the volume of fat in this will be the percentage by weight of fat multiplied by the specific gravity of the milk, divided by the specific gravity of the fat.

The milk-sugar may be calculated either as hydrated or as anhydrous sugar.

Official A.O.A.C. method

Determine the specific gravity of the milk. The quantity of sample to be taken for the determination varies with the specific gravity and is to be measured at the same temperature at which the specific gravity is taken. The volume to be measured will be found in Table 17.1, which is based on twice the normal weight of lactose (32·9 g per 100 ml) for the Ventzke sugar scale.

Table 17.1—Volumes of milk corresponding to a lactose double normal weight

Specific gravity of milk	Volume of milk for a lactose double normal weight (Ventzke scale)	Specific gravity of milk	Volume of milk for a lactose double normal weight (Ventzke scale)
1·024	64·25	1·031	63·80
1·025	64·20	1·032	63·75
1·026	64·15	1·033	63·70
1·027	64·05	1·034	63·65
1·028	64·00	1·035	63·55
1·029	63·95	1·036	63·50
1·030	63·90		

Place the quantity of milk indicated in the table in a flask graduated at 102·6 ml. Add 1 ml of the acid mercuric nitrate solution (see Vieth's method, *above*), fill to the mark, shake, filter through a dry filter, and polarise. It is not necessary to heat before polarising. If a 200 mm tube is used, divide the polariscope reading by 2 (or, if a 400 mm tube is used, by 4) to obtain the percentage of lactose in the sample.

Society of Public Analysts' method for determination of sucrose in sweetened condensed milk (Soc. Publ. Anal. 1930)

This method is recommended for the determination of sucrose in sweetened condensed milk, but by suitable modification it can be used for the determination of sucrose and lactose in fresh milk (see below). The method is described here primarily because it includes the most accurate correction for the volume of precipitate obtained on clarification; it also serves to illustrate the principle of the Clerget inversion and double polarisation.

The method for sweetened condensed milk is as follows—

REAGENTS—Zinc acetate solution: 21·9 g of crystallised zinc acetate $Zn(C_2H_3O_2)_2, 2H_2O$, and 3 ml of glacial acetic acid, in water, made up to 100 ml.

Potassium ferrocyanide solution: 10·6 g of crystallised potassium ferrocyanide in water made up to 100 ml.

Hydrochloric acid solution: 6·34 N.

Concentrated ammonia solution: nominal 0·880.

Dilute ammonia solution: 10 ml of concentrated ammonia solution diluted with water to 100 ml.

Dilute acetic acid solution approximately equivalent to the dilute ammonia solution.

APPARATUS—The instrument used for measuring the optical rotation may be either a polarimeter or a saccharimeter, using, for the polarimeter, sodium light or the green line of the mercury spectrum, separated by means of a prism or by the use of a special Wratten screen No. 77A, and for the saccharimeter white light from an incandescent electric lamp, after passing through 15 mm of a 6 per cent solution of potassium bichromate.

Tubes of not less than 2 dm, exactly calibrated for length.

Flasks and pipettes accurately calibrated in millilitres.

A standardised thermometer, reading to 0·1° C.

Preparation of the sample. Mix the sample by hand, using a spoon with an up-and-down rotary movement, in such a way that the top layers and the contents of the lower corners of the containing vessel are moved and mixed, care being taken that any separated crystals in the original sample should first be ground and incorporated in the bulk. It is important that frothing or the formation of air bubbles should be avoided.

PROCEDURE. Transfer to a 100 ml beaker an accurately weighed quantity, approximately 40 g, of the well-mixed sample; add 50 ml of hot distilled water (80° to 90° C), mix, transfer to a 200 ml measuring flask, washing in with successive quantities of distilled water at 60° C, until the total volume is from 120 to 150 ml. Mix, cool to air temperature, and then add 5 ml of the dilute ammonia solution. Again mix, and allow to stand for 15 minutes. Add a sufficient quantity of the dilute acetic acid solution to neutralise the ammonia added (the exact equivalent is determined beforehand by titration), and again mix. Add, with gentle mixing, 12·5 ml of zinc acetate solution and mix, followed in the same manner by 12·5 ml of potassium ferrocyanide solution. Bring the contents of the flask to 20° C and add distilled water at 20° C up to the 200 ml mark.

Up to this stage, all additions of water or reagents should be made in such a manner as to avoid formation of air bubbles, and, with the same object in view, all mixings should be made by rotation of the flask rather than by shaking. If bubbles are found to be present before completion of dilution to 200 ml, their removal can be assisted by temporary attachment of the flask to a vacuum pump, and rotation of the flask.

Close the flask with a dry stopper and mix thoroughly by shaking. Allow to stand for a few minutes and then filter through a dry filter-paper, rejecting the first 25 ml of filtrate.

Direct polarisation. Determine the rotation of the filtrate at 20·0° C.

Inversion. Pipette 40 ml of the filtrate obtained as above into a 50 ml flask; add 6 ml of 6·34 N hydrochloric acid. Immerse for 12 minutes the entire bulb of the flask in a water-bath maintained at 60° C, mixing by rotatory movements during the first 3 minutes, in which time the contents of the flask should have attained the temperature of the bath. Cool, dilute to 50 ml at 20° C with distilled water, mix, and allow to stand for one hour.

Invert polarisation. Determine the rotation at 20° C.

CALCULATION:

W = weight of sample taken, in grams.
F = percentage of fat in the sample.
P = percentage of protein (N × 6·38) in the sample.
V = volume to which the sample is diluted before filtration.
v = correction in ml for volume of precipitate produced during clarification.
D = observed direct polarimeter reading.
I = observed invert polarimeter reading.
l = length in dm of polarimeter tube.
Q = inversion divisor factor.

Then

$$v = \frac{W}{100}\left[(F \times 1{\cdot}08) + (P \times 1{\cdot}55)\right]$$

and percentage of sucrose in the sample

$$= \frac{D - (\frac{5}{4} \times I)}{Q} \times \frac{V - v}{V} \times \frac{V}{l \times W}$$

The inversion divisor factor Q. The value of Q in the above equation depends on the type of polarimeter, the source of light, the temperature, the concentration of the sugars in solution, and the effect of salts and acids in the solution. By working on solutions having the sugar, etc. concentrations ordinarily met with in applying the process to sweetened condensed milk, the following values for Q, all measurements being taken at 20° C, were obtained—

(*a*) Sodium lamp	0·8825
(*b*) Mercury green line (prism or special Wratten screen, No. 77A)	1·0392
(*c*) International Sugar Scale (*j*) light ..	2·5490

Where the concentration or temperature differs from those recommended, the corresponding values of Q are—

(*a*) $0{\cdot}8825 + 0{\cdot}0006\,(c - 9) - 0{\cdot}0033\,(T - 20)$
(*b*) $1{\cdot}0392 + 0{\cdot}0007\,(c - 9) - 0{\cdot}0039\,(T - 20)$
(*c*) $2{\cdot}5490 + 0{\cdot}0017\,(c - 9) - 0{\cdot}0095\,(T - 20)$

where c is the percentage of total sugars in the inverted solution as polarised, and T is the temperature at which the inverted solution is polarised; even when this correction is applied, T should always lie between 18° and 22° C.

Application of the method to fresh milk

One hundred and twenty grams of milk may be used, and then proceed without further dilution as in the Standard Method, by the addition of 5 ml of the dilute ammonia solution, etc.

In order to arrive at the percentage of lactose, the percentage of sucrose found is multiplied by $\frac{66\cdot5}{100\cdot0}$ to give the rotation due to the sucrose. This figure is subtracted from the specific rotation of the solution before inversion (corrected for the volume of precipitate) to give the rotation due to the lactose, and this is multiplied by $\frac{100\cdot0}{52\cdot5}$ to give the percentage of lactose hydrate in the sample.

With milk containing no added sucrose, the rotation observed before and after inversion, after allowing for dilution on inversion, should be equal, and the percentage of lactose hydrate in the sample

$$= \frac{D}{\cdot525} \times \frac{V - v}{V} \times \frac{V}{l \times W}.$$

(B), (C) GRAVIMETRIC AND VOLUMETRIC METHODS INVOLVING THE USE OF COPPER SOLUTIONS

Determination of sugars by copper reduction

When a polarimeter is not available, both volumetric and gravimetric methods based on the reduction of Fehling's solution may be used. The methods are empirical and require stringent control of the experimental conditions to obtain concordant results; furthermore, the amount of reduction obtained is not always directly proportional to the amount of the sugar present, which cannot be arrived at by simple calculation but must be obtained by the use of tables. Among the factors which may influence the results are: the presence of proteins, calcium, and lead in solution; the effect of non-reducing sugars (sucrose) on the reducing power of the reducing sugars; the fact that the reducing power of a mixture of sugars is not necessarily the sum of the reducing powers of the sugars present; the concentration of sugar present; and the effect of the precipitant used in preparing the sugar solution for the reduction process.

Before determining the milk-sugar in milk, the fat and protein must be removed; this may be accomplished by any of the following methods, although the first is recommended—

(1) Wash 25 g of the milk into a 250 ml graduated flask with about 50 ml of water, add 5 ml of zinc acetate solution and 5 ml of potassium ferrocyanide solution as used in the S.P.A. Polarimeter Method (*above*, p. 372), mixing gently after each addition; dilute to 250 ml and filter.

(2) Dilute 10 ml of milk to about 100 ml, add 1·5 ml of 10 per cent acetic acid solution, and boil; after cooling, make up to 100 ml and filter. (Citric acid may be substituted for acetic acid.)

(3) Add to 25 ml of milk about 200 ml of water and 10 ml of Fehling's copper sulphate solution; neutralise carefully with dilute caustic alkali solution and make up to 250 ml. Filter. This solution contains a small amount of copper.

(4) Neutralise carefully 10 ml of the filtrate from the milk, which has been treated with acid mercuric nitrate, with caustic alkali till exactly neutral to phenolphthalein; filter, and pass sulphuretted hydrogen through the filtrate; filter to separate the precipitated mercuric sulphide, and boil the filtrate to expel sulphuretted hydrogen. Make up to 100 ml. (Alternatively, the mercury may be precipitated by phosphoric acid; add a small quantity of phosphoric acid or a soluble phosphate to the filtrate from mercuric nitrate; neutralise exactly, filter and wash the precipitate and make up to 100 ml.)

Each of the methods of separating the fat and protein gives a solution of which 50 ml contains the milk-sugar in 5 ml of milk, which, in normal milk, represents about 0·24 g of milk-sugar.

Fehling's solution method (Soxhlet's modification)

(*a*) Dissolve 34·639 g of pure crystallised copper sulphate (molecular wt. 249·71) in water and dilute to 500 ml.

(*b*) Dissolve 173 g of pure sodium potassium tartrate (Rochelle salt) and 51 to 55 g of pure sodium hydroxide in water and make up to 500 ml. Equal parts by volume of these solutions are mixed (preferably at the time of making the test) to form Fehling's solution.

It is convenient to use a 50 per cent solution of caustic soda which has been filtered clear through asbestos for making up the alkaline tartrate solution. The percentage of sodium hydroxide in this is determined by titration, and such a quantity weighed out as will give 51 g. Most of the impurities in ordinary caustic soda are insoluble in a 50 per cent solution, so that this affords a ready means of purification.

Volumetric determination—Lane and Eynon's method

The modification of the Fehling's solution method usually adopted is that due to Lane and Eynon (1923, 1927). A one per cent aqueous solution of methylene blue is used as internal indicator, and dilution of the sugar solution should be such that between 15 and 50 ml are required to reduce the copper in either 10 or 25 ml of the mixed Fehling's solution. It is important that, in addition to the removal of protein and fat as mentioned above, the solution should be free from calcium or lead salts; these may be removed by means of potassium oxalate, and an excess of this reagent will not interfere with the subsequent titration.

The standard method, which should be rigidly adhered to, is as follows.

PROCEDURE. Ten or 25 ml of the mixed Fehling's solution are measured into a flask of 300 to 400 ml capacity and treated cold with almost the whole of the sugar solution required to effect reduction of all the copper, so that, if possible, not more than 1 ml is required later to complete the titration. The approximate volume of the sugar solution required is ascertained by a preliminary incremental titration which is described below. The flask containing the cold mixture is heated over a wire gauze; after the liquid has begun to boil it is kept in moderate ebullition for 2 minutes and then, without removal of the flame, 3 to 5 drops of the methylene blue indicator are added and the titration is completed in one minute further, so that the reaction liquid boils altogether for 3 minutes without interruption. The amount of sugar present in the number of ml used in the titration is then obtained from the Lane and Eynon sugar tables (pp. 554 to 559). The point of complete decolorisation of the methylene blue can usually be determined to within one drop of the sugar solution.

Since the volume of sugar solution required must be known approximately in order that almost the whole of it may be added at one time before boiling, a preliminary titration is usually necessary. This is carried out by Lane and Eynon as follows—

Incremental method of titration. Ten or 25 ml of mixed Fehling's solution in a 300 to 400-ml flask are treated cold with 15 ml of the sugar solution, and without further dilution, heated to boiling over a wire gauze. After the liquid has been boiling for about 15 sec. it is possible to judge if the copper is almost all reduced by the bright-red colour imparted to the boiling liquid by the suspended cuprous oxide. If it be judged that nearly all the copper is reduced, a few drops of the methylene blue indicator are added, boiling is continued for one to two minutes from the commencement of ebullition, and then the sugar solution is added in small quantities, say 1 ml or less at a time, the liquid being allowed to boil for about 10 sec. between successive additions until the colour of the indicator is completely discharged. If, after the mixture of Fehling's solution with about 15 ml of sugar solution has been boiling for about a quarter of a minute, there appears to be still much unreduced copper, a further 10 ml of sugar solution are added and the whole allowed to boil for a quarter of a minute, and so on, until it is considered unsafe to add a further large increment of sugar solution; boiling is then continued for one to two minutes, after which the indicator is added and titration is completed by small additions of the sugar solution. It is advisable not to add the indicator until the neighbourhood of the end-point has been reached, as the indicator retains its full colour until this point is almost reached, and gives no warning to the operator to go slowly. As in the standard titration, the aim is to give the reaction liquid about three minutes' total ebullition.

In general, a titration is made by the incremental method and then checked by the standard method.

Correction for sucrose. The presence of sucrose in sweetened condensed milk affects the determination of lactose by the Fehling's solution method. Lane and Eynon have investigated the magnitude of the errors due to this source when lactose is determined by their standard method. The effect of sucrose is to reduce the volume of lactose solution required by a given volume of Fehling's solution, and it may therefore be allowed for by adding an appropriate volume correction to the burette reading. The corrected volume may then be used to find the correct lactose content of the solution by reference to the standard lactose table.

The corrections vary in different parts of the table and also depend on the relative amounts of lactose and sucrose present. The following table, due to

Lane and Eynon, gives the corrections in mls for sucrose/lactose ratios of 3/1 and 6/1. It will be seen that at any given part of the table the correction is almost proportional to the sucrose/lactose ratio; this holds up to a ratio of about 10/1, so that for any ratio less than this the corresponding correction can readily be estimated.

Table 17.2—Corrections in ml to be added to burette readings in titration of lactose solutions containing 3 or 6 times as much sucrose as lactose

Volume of sugar solution required (ml)	10 ml Fehling's solution: ratio of sucrose to lactose		25 ml Fehling's solution: ratio of sucrose to lactose	
	3 : 1	6 : 1	3 : 1	6 : 1
15	0·15	0·30	0·30	0·60
20	0·25	0·50	0·30	0·60
25	0·30	0·60	0·35	0·65
30	0·35	0·70	0·35	0·70
35	0·40	0·80	0·40	0·80
40	0·45	0·90	0·45	0·90
45	0·50	0·95	0·55	1·10
50	0·55	1·05	0·60	1·20

Sucrose in sweetened milk products may also be determined with Fehling's solution by inverting a portion of the solution after the determination of the lactose, and again determining the total reducing power after suitably diluting the solution. The additional copper-reducing power due to the inversion, calculated as invert sugar and then multiplied by 0·95, gives the amount of sucrose originally present.

The inversion may be carried out as follows. To 50 ml of the sugar solution is added 5 ml of concentrated HCl, and the whole is heated in a water-bath at 68° C for 5 minutes. The solution is cooled, neutralised with solid sodium bicarbonate, added carefully to avoid undue frothing, and finally diluted so that between 15 and 50 ml are required to reduce 10 or 25 ml of Fehling's solution. The necessary dilution can only be determined in the first place by carrying out rough incremental titrations.

Pavy's-solution method for lactose in milk

Pavy's ammoniacal cupric solution may also be used to determine lactose in milk. It is prepared by mixing 120 ml of Fehling's solution, 400 ml of 12 per cent caustic soda solution, and 300 ml of strong ammonia (specific gravity 0·880) and diluting the whole to one litre.

A diluted solution of milk, which need not be clarified, is made by taking 10 g of milk and adding 45 ml of ammonia solution (specific gravity 0·90) and making up to 100 ml.

One hundred ml of Pavy's solution are placed in a 300-ml flask which is closed by a rubber stopper with two holes; through one passes the jet of a burette, and through the other a bent tube which dips into a flask containing cold water to absorb the ammonia given off. The solution is brought to the boil and the milk solution run in gradually till the blue colour of the liquid is destroyed, the boiling being maintained the whole time and the sugar solution run in slowly towards the end.

As the reaction takes place somewhat slowly, boiling must be continued for a few minutes before it can be finally decided that the blue colour is permanent.

It is necessary to repeat the titration, adding a little less of the solution, as with Fehling's solution. This may be added advantageously all at once, and the boiling continued for five minutes. If the boiling be prolonged unduly, the ammonia may be boiled off, and cuprous oxide will then begin to deposit; in order to avoid this, Shenstone places a tapped funnel in the cork, by means of which an addition of strong ammonia can be made if necessary.

Stokes and Bodmer strongly recommend this method, and state that the reducing power of milk-sugar is 52 per cent of that of glucose, i.e. 100 ml of Pavy solution = 0·0961 g of milk-sugar.

It is advisable to standardise the Pavy's solution on a solution of pure milk-sugar containing 0·5 g per 100 ml.

Hehner showed that by varying the proportion of salts in solution, such as alkaline tartrates and carbonates, the accuracy of the results is affected; but by standardising the solution at the time of using with a solution of pure milk-sugar, the effect of any such variation is eliminated.

Allen has modified the procedure by placing a layer of petroleum over the Pavy solution, and dispensing with the cork. This enables an ordinary burette, or even pipette, to be used.

A semi-micro method is due to Folin and Denis (1918), who dilute the 25 ml of milk to 100 ml with water, without previously removing protein or fat, and then take 2·8 to 3·4 ml of the diluted milk (i.e. nearly the full amount expected), 5 ml of copper sulphate solution (60 g crystallised copper sulphate and 4 ml concentrated sulphuric acid per litre), and 4 to 5 g of a dry salt mixture (100 g di-sodium hydrogen phosphate, 60 g sodium carbonate, and 30 g sodium or potassium thiocyanate) in a large test-tube and boil for four minutes with a small pebble to prevent bumping. The diluted milk is then added a few drops at a time, with boiling for one minute each time, until the liquid is just colourless. The total boiling period should be from 5 to 7 minutes. The volume of diluted milk required to bring about complete decolorisation contains 40·4 mg of lactose.

Gravimetric Fehling methods

Still one of the standard gravimetric methods is that due to Brown *et al.* (1897) and it depends on the determination of the amount of cuprous oxide precipitated on boiling the sugar solution with Fehling's solution. It may be carried out on a solution of milk which has been cleared of protein and fat by one of the methods previously described as suitable for copper reduction processes. It is important to adopt uniform conditions of precipitation, and the following conditions should be strictly adhered to, bearing in mind that it is also desirable to use an amount of sugar solution which will give a precipitate of copper oxide weighing between 0·1 and 0·3 g.

Place 50 ml of the mixed Fehling's solution (see Soxhlet's Modification, *above*) into a 250-ml squat-form beaker, add such an amount of water that, with the sugar solution to be added subsequently, the total volume will be 100 ml; cover the beaker with a clock-glass and heat for five minutes in a boiling water-bath, taking care that the height of the water outside the beaker is at the level of the solution inside, and that the beaker does not rest on the bottom of the bath. Then add the accurately-measured quantity of the sugar solution and continue the heating for exactly twelve minutes. The precipitate of cuprous oxide is then rapidly filtered off, washed with water and weighed as Cu_2O, CuO or Cu. The most convenient plan is to filter through a Gooch crucible and ignite the Cu_2O in air to CuO and weigh as the latter.

The amount of lactose or other sugar corresponding to the CuO is found by consulting the Tables (Elsdon 1923) on p. 560.

Alternatively, the precipitate of cuprous oxide may be filtered off, by the aid of a small funnel and a water-pump, through a hard glass tube about 1 cm wide, constricted near one end and plugged with asbestos which has been previously ignited in a current of hydrogen and weighed. The precipitate of cuprous oxide is well washed with boiling water, then with a few ml of alcohol; dried in an oven for a few minutes, ignited in a current of hydrogen for five minutes, cooled while still in the hydrogen, and the weight of copper then ascertained.

A blank experiment, using 50 ml of the mixed Fehling's solution, should not yield more than 0·5 mg of Cu_2O.

A.O.A.C. gravimetric method

The official Gravimetric Method for Milk of the A.O.A.C. is as follows—

Dilute 25 g of the sample with 400 ml of water in a 500-ml volumetric flask and add 10 ml of copper sulphate solution (Fehling's solution A) and about 7·5 ml of a KOH solution of such strength that one volume is just sufficient to precipitate completely the copper as hydroxide from one volume of the copper sulphate solution. Instead of KOH solution of this strength, 8·8 ml of 0·5 N NaOH solution may be used. After the addition of the alkali solution, the mixture must still have an acid reaction and contain copper in solution. The flask is then filled to the mark, mixed, and the liquid filtered through a dry filter. Fifty ml of the mixed Fehling's solution and 50 ml of the sugar solution (if a less quantity of sugar solution is used, the final volume should be made to 100 ml with water) are placed in a 400-ml beaker which is then covered with a clock-glass and heated on an asbestos gauze by a Bunsen burner so that it boils in 4 minutes and is allowed to boil for 2 minutes (6 minutes' heating in all). Filter at once through an asbestos-lined Gooch crucible with the aid of suction, and wash the precipitate thoroughly with water heated to about 60° C, then with 10 ml of alcohol, and finally with 10 ml of ether. Dry for 30 minutes in a 100° C oven, cool, and weigh the Cu_2O.

Alternative methods of determining the amount of the reduced copper are also given; of these, a modification of the Bertrand method is frequently adopted, the details being as follows.

Filter and wash the precipitate of Cu_2O with water as described above, transfer the asbestos pad and precipitate to a beaker, add about 30 ml of hot water and beat the precipitate and asbestos thoroughly. Rinse the crucible with 50 ml of a hot saturated solution of $Fe_2(SO_4)_3$ in 20 per cent H_2SO_4, the rinsings being received into the beaker containing the precipitate. When the copper precipitate is dissolved, wash the solution into a conical flask and immediately titrate the reduced iron with a standard solution of $KMnO_4$, 1 ml of which should be equal to 0·01 g of copper. The solution may be standardised against pure sodium oxalate acidified with sulphuric acid.

Barfoed's reagent

This reagent consists of a solution of copper acetate and acetic acid, is only slightly reduced by maltose and lactose, but is readily reduced by dextrose, laevulose, etc., and it can therefore be used to detect, and even to estimate, the amounts of the latter sugars present with lactose in milk products. Monier-Williams (1930) and the Milk Products Sub-Committee of the Society of Public Analysts (1932) have both investigated this reagent in connection with the detection and determination of invert sugar in sweetened condensed milk. The latter investigators found, however, that a quantitative determination of the amount of invert sugar could not be obtained by this method because of the condensation of part of the laevulose to laevan. They recommended, however, that a modified Barfoed reagent should be used to detect the formation of reducing sugars from the sucrose in sweetened condensed milk.

Laevan is a gum which is formed from nascent laevulose, resulting from the inversion of sucrose, by the action of the levanase of certain micro-organisms. Laevan does not reduce cupric solutions, and its $[\alpha]_D^{20°}$ is $-50°$, being less than that of laevulose. By the action of levanase, nine parts by weight of laevan are formed from ten parts of laevulose; by acid inversion, laevan is hydrolysed quantitatively to laevulose.

The Society of Public Analysts' Report shows that by modifying the composition of the copper acetate solution, reducing the temperature, and limiting and fixing the time for the reduction, the reducing action of milk serum free from invert sugar can be rendered negligible. Owing to the great sensitivity of the reaction to changes in condition, it is necessary, for accurate work, to standardise the reagent by the use of control sera containing approximately the same amounts of sugars as the test serum, and to heat them in the same bath as the latter.

The modified Barfoed solution will detect as little as 0·1 per cent of invert sugar, dextrose or laevulose in a condensed milk.

Society of Public Analysts' method

The serum used is obtained by treating approximately 40 g of the accurately weighed condensed milk with zinc acetate, etc., making to 200 ml and filtering, exactly as for the S.P.A. method for the polarimetric determination of sucrose described on p. 371.

REAGENTS—Copper solution—Dissolve 60 g of crystallised sodium acetate in water, add 105 ml of N acetic acid and make up to 1 litre with water. Transfer to a dry bottle, add 52 g (or more) of finely-powdered crystallised copper acetate, and shake to saturation. Filter.

Ferric sulphate solution—Dissolve 50 g of ferric sulphate in about 400 ml of water, to which 109 ml of concentrated sulphuric acid has been added. Make up to 1 litre with water and filter. Before use this solution should be treated with 0·1 N permanganate until the colour of the latter ceases to be discharged.

0·1 N potassium permanganate solution.

PROCEDURE—Introduce 25 ml of serum (zinc serum) into a thin-walled boiling-tube (internal measurements 8 by 1¼ in.); add 70 ml of the copper solution; mix; cover the tubes with a watch-glass and immerse, to the level of the liquid in the tube, in the water in a large water-bath maintained at 80° C for 20 minutes. Remove; cool in running water; filter on asbestos by suction and wash the tube and filter containing the cuprous oxide rapidly a few times with freshly-boiled distilled water, rejecting the filtrate and washings. Dissolve the cuprous oxide (including any remaining in the tube) in 20 ml of the ferric suphate solution; wash the asbestos pad with cold, freshly-boiled distilled water, adding the washings to the ferric sulphate filtrate, and titrate with 0·1 N permanganate to faint permanent pink.[1] In the absence of laevulose, dextrose and invert sugar, the titration will only be of the order of 0·15 ml of 0·1 N $KMnO_4$.

Note—A convenient asbestos filter may be prepared by inserting a loosely-fitting glass bead into the neck of an Allihn filter-tube; above the bead is placed a layer of glass wool, and on this is laid the asbestos, which is added as a wet pulp and drawn on to the glass wool by suction. Solution of the cuprous oxide is rapidly effected by pipetting the ferric sulphate solution into the reduction tube, transferring to the Allihn tube and thoroughly mixing the surface asbestos layers with the sulphate solution by means of a flat-ended glass rod, leaving the lower asbestos layers and the glass wool undisturbed.

(D) IODOMETRIC AND OTHER METHODS

Oxidation of sugars by iodine was first investigated by Romijn (1897) and has since attracted the attention of many workers. Particular mention should be made of the investigations conducted by Hinton and Macara (1924, 1927, 1931) using both alkaline iodine solutions and solutions of chloramine-T as the oxidising agent. The methods suggested in these latter papers have been adopted in the report of the Milk Products Sub-Committee of the Society of Public Analysts on the analysis of sweetened condensed milk in which the sucrose has altered during storage (Soc. Publ. Anal. 1932).

Although oxidation by iodine can be used as an alternative to any of the methods of determining sugars previously mentioned, its chief merit lies in that it is possible by this means to determine laevulose in a mixture of other sugars. Alkaline iodine solutions have the property, at ordinary temperatures, of converting aldose sugars (i.e. those containing the group —CHO) into the corresponding monobasic acid; whereas ketose sugars (those containing the group =CO), such as laevulose, are almost unaffected. One gram of dextrose requires 1·41 g of iodine for oxidation, while a corresponding amount of the disaccharides maltose or lactose hydrate requires only half the quantity of iodine.

The experimental procedure recommended by Hinton and Macara is as follows. To 50 ml of test solution containing about 0·08 of dextrose, or its equivalent, is added 40 ml of 0·05 N iodine solution and 5 ml of 0·5 N sodium hydroxide solution; the flask is stoppered and allowed to stand 10 minutes at

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[1] If the titration exceeds about 15 ml 0·1 N $KMnO_4$, the test should be repeated with a suitable quantity of serum made up to 25 ml. For specially accurate work, the dilution should be made with a serum prepared from fresh milk and sucrose.

17·5° C and then acidified with 5 ml of 2 N sulphuric acid, and the excess of iodine at once determined with 0·05 N thiosulphate solution. Starch solution is used to determine the end-point, and blank determinations are carried out with each experiment.

In view of the reactive nature of iodine with non-sugar substances such as proteins, these tend to interfere; with pure sugar solutions, however, the following amounts of iodine, calculated as grams of iodine per gram of sugar, are absorbed—

Dextrose	1·410
Lactose hydrate	0·705
Invert sugar	0·710
Sucrose	0·003
Laevulose	0·012

The figure given for laevulose applies only when an equal amount of dextrose is present, i.e. in invert sugar; other proportions of dextrose modify the extent of the oxidation of the laevulose.

Invert sugar is particularly sensitive to the effect of acids, high results being obtained if it is heated in the presence of acids. For this reason the inversion of sucrose must not be carried out at a higher temperature than 60° C, and for not longer than 10 minutes. It therefore follows that the exact determination of invert sugar, particularly when it may have been previously in contact with acid, is difficult by this method and should be checked by a copper reduction.

With sucrose, it is apparent from the above table that under satisfactory conditions the iodine reduction of invert sugar formed from sucrose is 0·710 gram of iodine per gram of sugar; the sucrose before inversion gave a value of 0·003; therefore an increase in iodine reduction of $\frac{0{\cdot}707}{0{\cdot}950}$ is given per gram of sugar inverted. The factor for calculating sucrose from the difference between " direct " and " invert " iodine reduction is therefore 0·744.

Adaptation of the above reaction to the determination of laevulose in sweetened condensed milk

Hinton and Macara (1931) have developed a process from the observations of Kolthoff (1922) (which were elaborated by Kruisheer 1929), and which consists essentially in the oxidation of lactose and dextrose by alkaline iodine solution and the determination of the remaining laevulose by copper reduction. In order to avoid the interference of sucrose in the copper reduction which occurs when Fehling's solution is used, Kruisheer used Luff's copper reagent, which is unaffected by sucrose. In the determination of cuprous oxide the method of Shaffer and Hartmann (1921) has been adopted, and it has the advantage that no separation of cuprous oxide from the excess of the copper reagent is required.

As previously mentioned in the special case of sweetened condensed milk, the formation of invert sugar is also attended by the formation of laevan from a portion of the laevulose, with the result that the determination of the latter cannot be used to arrive directly at the amount of invert sugar present. The determination of the laevulose present as such is, however, necessary in any scheme for the complete analysis of condensed milk.

Hinton and Macara's process has been adopted by the S.P.A. Sub-Committee (1932) for the determination of laevulose in sweetened condensed milk. The process consists of four stages: (*a*) preparation of the serum, the zinc acetate method being used exactly as for the S.P.A. method for the polarimetric determination of sucrose in condensed milk; (*b*) oxidation of lactose and dextrose by alkaline iodine solution; (*c*) reduction of copper (Luff's solution) by the laevulose; and (*d*) determination of the reduced copper by Schaffer and Hartmann's iodometric method. The following are the details of the process as given in the S.P.A. Report—

REAGENTS. Sucrose solution—Approximately 9 g per 100 ml (freshly prepared).

Iodine solution—13 g of iodine and 15 g potassium iodide per 100 ml.

Mixed alkali solution—Equal parts of 2N sodium carbonate and 2N sodium hydroxide.

Sulphuric acid—Approximately 5N.

Sodium sulphite solution—20 per cent wt/vol.

Dilute sodium sulphite solution—2 per cent, freshly prepared; or diluted from the 20 per cent solution.

Luff's solution. Dissolve 25 g of crystallised copper sulphate in 100 ml of water; 50 g of citric acid in 50 ml of water; 388 g of $Na_2CO_3,10H_2O$ in 300 or 400 ml of lukewarm water. Add the citric acid solution to the sodium carbonate solution, and then add the copper solution. Mix, cool, make up to 1 litre, and filter.

> *Note*—This solution should be accurately prepared, and 10 ml of the finished solution should require approximately 45 ml of 0·5 N sulphuric acid for neutralisation to methyl orange.

Iodate-iodide solution—2·7 g of potassium iodate, 30 g of potassium iodide, and 10 ml of 0·5 N sodium hydroxide solution per litre.

Potassium oxalate solution—A saturated aqueous solution.

Sodium thiosulphate solution—0·05N.

Soluble starch solution—Approximately 2 per cent.

Control serum—Prepared from fresh milk, using 100 ml of milk with the same quantities of ammonia and acetic acid and precipitants as for 40 g of condensed milk, made up to 200 ml and filtered.

PROCEDURE—(i) *Oxidation of the aldose sugars.* Pipette 10 ml of the prepared condensed milk serum (zinc serum) and the same amount of the control serum into 250-ml conical flasks, ensuring that the liquid does not flow on to the sides of the flasks. To the condensed milk serum add 10 ml of water, and to the control serum 10 ml of the sucrose solution.

> *Note*—Some workers find it preferable to add 2 ml of a 0·5 per cent solution of invert sugar to each serum so as to ensure a perceptible reduction in the control; this is termed " sensitising invert ". Such addition makes no difference to the subsequent procedure.

To each, then add exactly 5 ml of the iodine solution and exactly 6 ml of the 2 N mixed alkali solution; mix gently, and allow the flasks to stand for 10 minutes at from 18° to 20° C. Acidify with 1·6 ml of 5N sulphuric acid, and remove the liberated iodine, first with 20 per cent sodium sulphite solution and finally, after adding 6 drops of soluble starch solution, with the 2 per cent sulphite solution. (This operation should have the precision of a titration, though the quantities of sulphite solution needed are not measured; it should be conducted as rapidly as possible.) When all free iodine is eliminated, immediately add one drop of methyl orange solution and neutralise with the 2N mixed alkali solution.

Note—The time elapsing between acidifying with 5N sulphuric acid and neutralising with the mixed alkali should not exceed 5 minutes, to avoid the danger of inversion of the sucrose.

(ii) *Treatment with Luff's solution.* To the contents of each flask add 20 ml of Luff's solution; cover with a watch-glass and heat the contents to boiling on a plain wire gauze over a burner, regulated so that boiling takes place in 2 minutes; impinging of the flame or hot gases on the sides of the flask should be prevented by an asbestos sheet with central hole of suitable dimensions placed in contact with the wire gauze. When boiling takes place, transfer the flask to an asbestos-covered gauze already heated by a small Bunsen flame, attaching a reflux condenser, and maintain gentle ebullition for exactly 10 minutes.

Remove from the flame and cool in running water for four or five minutes.

Titration of the reduced copper. Add exactly 25 ml of the iodate-iodide solution and 20 ml of saturated potassium oxalate solution. Acidify carefully, while swirling, with 20 ml of 5N sulphuric acid. Shake round (with some care, as frothing occurs) until the precipitate of cuprous oxide (which is partly converted into white cuprous iodide) has dissolved, and titrate with 0·05 N thiosulphate. No further addition of starch should be required. The end-point is distinguished by a sharp change to a fine light blue (the colour of the cupric salt).

Calculation of the laevulose. The difference between the titrations of the sample serum and the control serum, as ml of 0·05 N thiosulphate solution, multiplied by 0·064, gives the percentage of laevulose in the sample, uncorrected for the volume of the clarification precipitate. (This factor is strictly correct only for a 20 per cent serum, i.e. if exactly 40 g of condensed milk were diluted to 200 ml in the preparation of the serum.) The correction for volume of precipitate, if required, is calculated as in the S.P.A. method for sucrose (p. 371) or " Original Sucrose " (*below*).

Chloramine-T and potassium iodide oxidation method

Results obtained by Hinton and Macara (1927) with the alkali-iodine method for lactose in condensed milk tended to be higher than those obtained by copper reduction, due apparently to the oxidation of non-sugar substances in the serum by the hypoiodite. It was also found that when neutral iodine solution was used, there was no oxidation of the non-sugar substances. For this reason a mixture of chloramine-T and potassium iodide was suggested as a more suitable oxidising agent for use with milk serum. Although the oxidation is then much slower (requiring 1½ hours for completion) it was found much easier to control, and as long as the reaction mixture is slightly alkaline—which can be, to some extent, automatically provided for if the iodide is added to the serum before the chloramine—the effect of the non-sugar milk constituents is almost entirely removed. Chloramine-T, in the presence of potassium iodide, forms hypoiodite and some free iodine, the hypoiodite being available to oxidise aldoses, as in the alkaline iodine method. The unaltered chloramine and hypoiodite at the end of the oxidation can be determined by acidification followed by a thiosulphate titration. One molecule of chloramine-T is equivalent to 2 atoms of iodine in both the oxidation and the final back-titration; the amount used up by the sugar is therefore conveniently expressed in terms of iodine.

It will be observed therefore that the chloramine-T and potassium iodide method and the alkaline iodine method are basically similar in that they both depend on oxidation by hypoiodite; and within limits imposed by the effect of various interfering substances such as proteins, sugars, etc., they both give similar results. Reference should be made to the original paper for details of the determination of lactose and sucrose by this method; tables of factors corresponding to the various amounts of chloramine used and, with lactose, corrections for the amount of sucrose present are given in the paper. The method of carrying out a chloramine titration is, however, described in the following section.

Determination of "original sucrose" in sweetened condensed milk

The method recommended by the Milk Products Sub-Committee of the Society of Public Analysts (1932) for the above determination, which is important when the amount of milk solids has to be determined in a sample of condensed milk in which the sucrose has partially decomposed into dextrose, laevulose, and laevan, includes a polarimetric reading and a chloramine-T titration. Details of the method are as follows.

The zinc serum used for the polarimetric reading is obtained as directed on p. 372.

Specific rotation of the inverted serum

REAGENT. Hydrochloric acid solution = 6·34 times normal.

Inversion. Pipette into a 50-ml measuring-flask 40 ml of the filtrate obtained by zinc clarification; add 6 ml of 6·34 N hydrochloric acid. Immerse the entire bulb of the flask for 12 minutes in a water-bath maintained at 60° C, mixing by rotary movement during the first three minutes, in which time the contents of the flask should have attained the temperature of the bath. Cool, and make up to the 50-ml mark at 20° C with distilled water; mix, and allow to stand for one hour.

Invert polarisation. Determine the rotation at 20° C.

W = weight of sample taken in grams
F = percentage of fat in the sample
P = ,, ,, protein ($N \times 6{\cdot}38$) in the sample
V = volume (in ml) to which the sample is diluted before filtration
v = correction in ml for volume of precipitate produced during clarification
I = observed invert polarimeter reading
l = length in dm of polarimeter tube
R* = specific rotation of the inverted serum $[\alpha]_D^{20}$.

*Note—If the mercury green line is used instead of the D line, the reading should be multiplied by 0·847 to convert to rotation for the D line.

Then,

$$v = \frac{W}{100}[(F \times 1{\cdot}08) + (P \times 1{\cdot}55)]$$

and

$$R = \left(\frac{5}{4} \times I\right) \times \frac{V - v}{V} \times \frac{V}{l \times W}.$$

Preparation of phosphotungstic acid serum for chloramine-T titration

REAGENT. *Phosphotungstic acid precipitant.* Fifty grams of crystalline sodium tungstate, $Na_2WO_4,2H_2O$, and 6 g of crystalline di-sodium phosphate are dissolved in about 200 ml of distilled water, and 220 ml of 2N hydrochloric acid solution (or the equivalent amount of acid of other normality) are added slowly with stirring. The solution is diluted to 500 ml and filtered. The acidity of the reagent should be so adjusted that 20 ml require approximately 16·0 ml of 0·5 N sodium hydroxide solution when titrated with methyl orange as indicator, and the pH of the reagent, diluted to five times its volume with water, is approximately 1·3.

PROCEDURE. Transfer to a 200-ml measuring-flask an accurately weighed quantity of 10 g of the well-mixed sample by successive quantities of distilled water at about 60° C, using about 120 ml of water in all. Mix; cool to air temperature, and add, with gentle mixing, 10 ml of the phosphotungstic acid reagent. Bring the contents of the flask to 20° C, and add distilled water (at 20° C) up to the 200-ml mark.

Up to this stage, all additions of water or reagents should be made in such a manner as to avoid formation of air bubbles.

Close the flask with a dry stopper and mix thoroughly by shaking. Allow to stand for 10 minutes, and then filter through a dry filter-paper, rejecting the first 25 ml of filtrate.

(With small samples, the full quantity given above may not be available. If the prescribed quantity of 10 g is departed from, however, the amount of the phosphotungstic acid reagent must be varied so that its volume in millilitres is numerically equal to the weight of sample taken in grams. The calculation for the phosphotungstic acid serum holds only when this relationship is maintained and for a 5 per cent serum.)

The chloramine-T titration

REAGENTS. 0·05 N chloramine-T solution—Containing 7·04 g per litre, freshly prepared, and protected from light.

Standard sodium thiosulphate solution—Preferably rather stronger than 0·05 N, so that 50 ml of the chloramine-T solution can be titrated without refilling a 50-ml burette.

> *Note*—The thiosulphate must be accurately standardised against pure potassium dichromate by the method of Popoff and Whitman (1925). The procedure is as follows—
>
> To 25 ml of 0·1 N potassium dichromate solution add 20 ml of 10 per cent potassium iodide solution and 10 ml of 2N hydrochloric acid; stopper the flask, and allow to stand in the dark for 10 minutes; then titrate with the thiosulphate.

0·5 N sodium hydroxide solution.
0·1 N sodium hydroxide solution.
Soluble starch solution—Approximately 2 per cent.

PROCEDURE. *Inversion.* Pipette 25 ml of the phosphotungstic acid serum (see above) into a 100-ml measuring-flask, add 15 ml of distilled water and 5 ml of 6·34 N hydrochloric acid. Immerse for 12 minutes the entire bulb of the flask in a water-bath maintained at 60° C, mixing by rotatory movement during the first 3 minutes, in which time the contents of the flask should have attained the temperature of the bath. Cool, add 0·5 N sodium hydroxide solution, continually mixing, until neutral, carrying the addition of alkali to the point of a definite turbidity (no internal indicator should be used). Cool and make up to the 100 ml mark at 20° C with distilled water.

Titration of inverted and neutralised serum. Into one of two 250 ml flasks or bottles pipette 25 ml of the inverted neutralised serum which has been diluted to 100 ml; into the other pipette 25 ml of water (as a blank). To each add 3 ml of 0·1N caustic soda solution, followed by 20 ml of 10 per cent potassium iodide solution; then, into each, pipette accurately 50 ml of the 0·05 N chloramine-T solution; close the flasks or bottles and leave in the dark for 1½ hours at a temperature of from 17° to 18° C. At the end of this time add to each flask 10 ml of 2N hydrochloric acid and titrate at once with the thiosulphate solution, with starch solution as indicator.

Note—All measuring-flasks, pipettes and burettes must be accurately calibrated, special attention being paid to drainage of the burette, which should be calibrated in the manner in which it is used for the titration. Readings of the burette are made to 0·01 ml.

CALCULATION:

S = percentage of " original sucrose " in the sample.
E = iodine absorbed per 100 grams of sample.
W = weight of sample taken in grams.
F = percentage of fat in the sample.
P = percentage of protein (N × 6·38) in the sample.
V = volume (in ml) to which the sample is diluted before filtration.
v = correction in ml for volume of precipitate produced during clarification.
n = difference between the inverted serum and the blank titrations in ml 0·05 N thiosulphate solution.

Then, for calculation of the original sucrose,

$$v = \frac{W}{100}[(F \times 1{\cdot}08) + (P \times 0{\cdot}74) + 3{\cdot}75] \text{ ml}$$

$$E = (n - 0{\cdot}15^*) \times 63{\cdot}46 \times 0{\cdot}032 \times \frac{V - v}{V}$$

and $$S = 0{\cdot}964\,E - 1{\cdot}29\,R.$$

Perry and Doan (1950) have described a simple, empirical, picric acid method for the simultaneous determination of sucrose and lactose in dairy products.

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* This is the correction for absorption by non-sugars; it applies only to a normal milk serum and for the quantities indicated in this determination.

(3) DETERMINATION OF CITRIC ACID IN MILK, CONDENSED MILK, ETC.

Present-day processes for the determination of citric acid in milk are usually based on its oxidation by potassium permanganate to acetone-dicarboxylic acid, which is then treated with bromine to form pentabromo-acetone. This method was originally used by Stahr (1915) for the determination of citric acid in wine. Numerous modifications of the method adopted for the determination of citric acid in milk have been published, including the following: Kunz (1916); *Methods of analysis*, A.O.A.C. (1950); Kometiani (1932); Hartmann and Hillig (1932); Lampitt and Rooke (1936); and Arup (1938).

Lampitt and Rooke's method

Lampitt and Rooke have made an exhaustive study of the methods available for the determination of citric acid in milk, and the following are the details of the pentabromo-acetone method as recommended by them—

REAGENTS. Sulphuric acid: 1 vol. concentrated acid (sp. gr. 1·84) + 1 vol. water.
Potassium bromide solution: 37·5 per cent wt/vol.
Potassium permanganate solution: 5 per cent wt/vol.
Ferrous sulphate solution: 20 per cent (crystals) wt/vol in 1 per cent sulphuric acid.
Phosphotungstic acid solution: Dissolve 20 g of phosphotungstic acid in water and dilute to 100 ml.

Preparation of the serum. One hundred and fifty g of milk are heated to 50° to 60° C in a 250-ml graduated flask and 25 ml of potassium oxalate solution (2 per cent) are added; contents of flask are shaken; 20 ml sulphuric acid (1 : 1) are added and contents of flask shaken; after cooling, 10 ml of phosphotungstic acid solution are added and contents are made up to 250 ml; after vigorous shaking, contents are allowed to settle for 5 minutes and the serum is filtered from the precipitate.

METHOD. To 50 ml of the milk serum, prepared as described above, or other solution containing citric acid, are added 10 ml of the sulphuric acid (if not already added in the preparation of the solution) and 5 ml of potassium bromide solution. (Except for pure citric acid and milk serum, 10 ml of freshly-prepared bromine water should also be added, and any precipitate formed from acetone-dicarboxylic acid filtered off after half-an-hour's standing.) Potassium permanganate solution is added drop-wise from a burette with constant shaking, until a brown precipitate persists, 10 ml being required usually for 0·1 g of citric acid and 25 ml for a milk serum. The mixture is allowed to stand at room temperature for 1 hour, further addition of permanganate being made if the brown precipitate disappears. Sufficient ferrous sulphate solution is then added slowly till a pale yellow solution containing a white precipitate is obtained, and the mixture is cooled in an ice-chest overnight (16 hours).

The precipitate is removed by filtration through a sintered glass crucible (size 10 G 4), the reaction flask being washed out with the filtrate to remove the last traces of precipitate, and the washings passed through the crucible.

The precipitate in the crucible is then washed with portions of 10, 10 and 5 ml of cold water. The crucible is dried to constant weight in a vacuum desiccator (about 16 hours). The precipitate is dissolved out of the crucible with industrial spirit followed by 20, 10 and 10 ml portions of ether. The crucible is again dried in the vacuum desiccator and weighed, the loss in weight being taken as pentabromo-acetone.

Then

$$\text{citric acid (anhydrous)} = 0{\cdot}424\left(W + \frac{0{\cdot}005V}{100}\right)$$

where W represents the difference in weight of the crucible before and after treatment with industrial spirit and ether, and V the original volume of filtrate from reaction mixture, less the total volume of washings.

Arup (1938) points out that while the above method is satisfactory for fresh milk it fails for sweetened condensed milk, malted milk, etc. This is due to the fact that in the presence of cane sugar and malt sugars the quantitative conversion of citric acid into pentabromo-acetone is not complete. Arup therefore suggests the following preliminary treatment of sweetened milk products.

Preparation of serum from sweetened condensed milk, malted milk, milk chocolate, etc. Weigh out 20 g of the sample in a wide-necked 100 ml flask (such as the Reichert type), add 60 ml of hot water, heat on the water-bath and mix thoroughly; add 8 ml of 50 per cent trichloracetic acid, mix and heat on the water-bath for half-an-hour, shaking occasionally. Cool, make up to 100 ml, filter and measure an aliquot portion of the filtrate (usually 75 ml) into a 100 ml beaker. Add phenolphthalein and then sufficient sodium hydroxide solution (at first a strong solution and finally 0·1 N) to produce a faint pink tint. Add an excess, usually 10 ml, of neutral lead acetate solution (15 per cent), mix, allow to settle and filter off the precipitate on a Gooch crucible with about 0·1 to 0·2 g of asbestos; after sucking dry, wash with 10 ml of water. It is not necessary to remove all traces of sugar or to transfer all traces of the precipitate to the filter. Transfer the crucible with the precipitate and asbestos to the beaker previously used, add 70 ml of hot water and 15 ml of 50 per cent (by volume) sulphuric acid, stir up the precipitate and asbestos in the acid, warm on the water-bath for 15 minutes with occasional stirring, transfer to a 100–110 ml measuring-flask, cool, make up to the 110-ml mark with water, and filter off 100 ml for the determination of citric acid, which will proceed normally after the treatment just described. As regards volume corrections for the precipitates, it was found that for the first precipitate the formula—

$$v = \frac{W}{100}\left[(1{\cdot}08 \times F) + (1{\cdot}55 \times P)\right]$$

where v represents the volume of precipitate in millilitres, W the weight of sample in grams, F the percentage of fat in the sample, and P the percentage of protein (N × 6·38)—gives sufficiently accurate results. For the second precipitate, with the quantities given, the volume of the lead sulphate and asbestos may be taken as 0·4 ml.

By the above method for fresh milk, Arup found that the results of citric acid determinations on 25 samples of milk varied from 0·150 to 0·206 per cent with an average of 0·168 per cent. These results obtained by a recent and modified method are in striking agreement with the figures of 0·15 to 0·20 per cent given by Richmond (1920).

The original *Stahr-Kunz pentabromo-acetone method* is briefly as follows—

Fifty ml of milk are treated with 20 ml of 50 per cent sulphuric acid, 2 ml of 40 per cent potassium bromide solution, and 20 ml of 10 per cent phosphotungstic acid, and diluted to 200 ml and filtered. To 150 ml of the filtrate are added 25 ml of freshly-prepared saturated hydrobromic acid, the solution is heated to 50° for five minutes, and then treated with 10 ml of 5 per cent potassium permanganate, being stirred the whole time. The precipitate, which consists of pentabromo-acetone, is collected, dried over sulphuric acid and weighed, the weight multiplied by 0·236 giving citric acid.

Two other methods of determining citric acid in milk are given in the following paragraphs—

Gowing-Scopes' method

Gowing-Scopes (1913) investigated the method originated by Denigès, which consists in oxidising citric acid to acetone-dicarboxylic acid, and the conversion of this into an insoluble basic mercury salt. He found that, to obtain exact results, strict attention must be paid to details of manipulation.

For the determination of citric acid in milk, the clear filtrate obtained by adding acid mercuric nitrate to milk as for the estimation of milk-sugar is used. Ten ml of this are neutralised exactly with alkali, using phenolphthalein as indicator; a precipitate will form, but this will be redissolved on the addition of 10 ml of a reagent prepared by covering 51 g of mercuric nitrate and 51 g of manganese nitrate with 68 ml of strong nitric acid, and after the addition of 100 ml of water to dissolve the salts, making up the volume to 250 ml and filtering.

This solution, made up to 200 ml, is boiled under reflux for three hours, filtered through a weighed Gooch crucible, and the precipitate washed well with cold water; the deposit on the sides of the flask may be removed by adding 1 or 2 ml of 1 per cent nitric acid and rubbing with a rod; after drying for five hours the precipitate is weighed. The colour of the precipitate should be nearly white; if it is yellow, this is due to the presence of basic salts, and the result will be high.

The weight of the precipitate multiplied by 0·1667 will give the amount of citric acid, and in calculating the percentage in the milk, the corrections for the volume of the precipitated proteins and fat must be made as in Vieth's method of milk-sugar estimation.

The method given above departs slightly from Gowing-Scopes' original method, as, owing to the presence of mercury in the filtrate, slightly more mercuric salts are present than he prescribes, but his researches have shown that variations of the amount of mercury used have far less influence on the results than variations in the other ingredients of his reagent.

Richmond's method (Richmond 1920)

The proteins are precipitated by acid mercuric nitrate (p. 370) and a measured volume of the clear filtrate neutralised exactly with dilute caustic soda solution, using phenolphthalein as indicator. A white precipitate of mercury and calcium phosphate and citrate is thrown down, collected on a filter, and washed with water; it is removed from the filter, suspended in water, and a little dilute hydrochloric acid added; sulphuretted hydrogen is passed through to precipitate the mercury as mercuric sulphide. After filtration, the solution is boiled to remove sulphuretted hydrogen, and, after the addition of a little calcium chloride, cooled. It is then neutralised carefully, phenolphthalein being used as indicator; the precipitate of calcium phosphate is filtered off, and the solution boiled and concentrated to a small bulk; the calcium citrate is thus precipitated. This should be washed with boiling water, collected on a small filter and ignited. To the ignited residue an excess of standard hydrochloric acid is added and the excess titrated back with standard alkali, methyl orange being the best indicator. Each millilitre of 0·1 N hydrochloric acid used represents 0·0064 g of citric acid.

The result must be corrected for the volume of the fat and protein thrown down as directed under milk-sugar.

Babad and Shtrikman (1951) have applied the method of Saffran and Denstedt to the estimation of citric acid in milk. This is based upon the yellow colour when pyridine in acetic anhydride reacts with citric acid. The accuracy of the method is considered to be ± 3 per cent. Using this method they have found values varying from 0·16 per cent to 0·25 per cent citric acid in whole milk.

(4) DETERMINATION OF LECITHIN–PHOSPHORIC ACID IN MILK

Lecithin is one of a series of mixed glycerides, containing both phosphorus and nitrogen, called "phosphatides", present in relatively large amounts in egg yolk and to a lesser extent in other animal and vegetable substances. According to Mohr *et al.* (1933), these compounds are present in milk and milk products to the following extent: milk 0·037 per cent, skim-milk 0·016 per cent, cream 0·169 per cent, and butter 0·206 per cent. Lecithin contains 3·84 per cent of phosphorus, equivalent to 8·8 per cent of P_2O_5. As a measure of the amount of phosphatides present in foods, it is usual to determine the amount of organically combined phosphoric acid which can be extracted by solvents and to return this figure as percentage of lecithin–phosphoric acid (P_2O_5).

A suitable quantity of the sample, usually 10 to 30 g, is powdered or dried with sand. It is then extracted, first with ether and then with alcohol, for 10 hours at a temperature within the extractor of at least 55° C. The combined ether and alcohol extracts are then evaporated with about 10 ml of 0·5 N alcoholic potash (or sufficient to saponify completely the fatty matter). The residue is transferred to a platinum dish, evaporated to dryness, and carefully charred.[1] It is then extracted with dilute nitric acid, filtered, washed, and the residue and filter returned to the dish and ashed. The ash is treated with nitric acid, filtered, and added to the first filtrate. The solution is boiled with excess of nitric acid, neutralised with ammonia, a few grams of solid ammonium nitrate added and the solution finally made just acid with dilute nitric acid;

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[1] Alternatively, instead of adding alkali and charring, organic matter may be destroyed by nitric and sulphuric acids.

after heating to 70° C the phosphate is precipitated with excess ammonium molybdate solution (see " Analysis of commercial casein ", p. 476). After allowing to settle for 1 hour the solution is filtered through paper pulp at the pump, the precipitate washed first with 1 per cent nitric acid and then with 1 per cent potassium nitrate, until free from acid. The paper pulp and precipitate are transferred bodily to a known excess of 0·1 N caustic soda, and after the precipitate has dissolved, the excess alkali is titrated back with 0·1 N hydrochloric acid to phenolphthalein. Each ml of 0·1 N caustic soda used by the precipitate is equivalent to 0·00031 g P_2O_5.

Bordas and de Raczkowski determined lecithin in milk by the following process. One hundred ml of milk are shaken with 100 ml 95 per cent alcohol, 100 ml of water and 10 drops of acetic acid. The precipitate is extracted with three successive quantities of 50 ml each of hot absolute alcohol. The mixed extracts are evaporated, and the residue taken up with a small quantity of a mixture of equal parts alcohol and ether; the ether is then evaporated, the residue saponified by potassium hydroxide, and the soap solution acidified with dilute nitric acid. The fatty acids are filtered off, and the filtrate evaporated to dryness, mixed with 10 ml of strong nitric acid, and oxidised completely by the addition of potassium permanganate little by little. A few drops of sodium nitrite solution (1 : 10) are added to dissolve the manganese hydroxide, and the nitrous acid expelled by boiling. The phosphoric acid is precipitated with ammonium molybdate and determined as magnesium pyrophosphate.

CHAPTER 18

THE DETECTION AND DETERMINATION OF PRESERVATIVES IN MILK

DETECTION OF NITRATES, ADDED COLOURING-MATTER AND SUCROSE

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(1) THE DETERMINATION OF PRESERVATIVES

The addition of any preservative to milk is prohibited in this country. Substances which have been used in the past for their preservative action include: boric acid and borax, formaldehyde, benzoic acid, salicylic acid, fluorides, hydrogen peroxide, hypochlorite solutions, β-naphthol, nitrites, etc.

In addition to the above substances, which are used for their bactericidal action, alkalis, such as sodium carbonate or sodium bicarbonate, may be added to neutralise the acidity formed during souring and thereby to delay the curdling of the milk. An examination of the soluble ash would quickly prove the presence or otherwise of any appreciable quantity of alkali carbonate.

Boric acid and borates

These are readily detected in milk by placing a few drops (0·5 ml) of the sample in a depression on a spot plate, adding 1 drop of turmeric tincture and 2 drops of 0·2 N hydrochloric acid. After mixing the solution with a glass rod, the tile is warmed on the water-bath until the milk is dry. In the presence of boric acid, a pink colour will be developed which is most marked just as the mixture becomes dry; a drop of sodium bicarbonate solution changes the colour to olive-green. The test will detect the presence of 0·005 per cent of boric acid.

Alternatively, boric acid may be detected by acidifying some of the milk with dilute hydrochloric acid, dipping a turmeric paper into the mixture, and drying the paper on a watch-glass in an oven at 100° C. On drying, the paper will assume a pinkish-brown coloration, turned a very dark green—almost black—on moistening with a solution of sodium bicarbonate. Cribb and Arnaud prepare turmeric paper by boiling 2 g of turmeric and 2 g of tartaric acid with 80 per cent alcohol till the latter is dissolved, and soaking strips of filter-paper in this solution. It is very delicate, and should be kept in the dark. Another test is to moisten the ash with dilute sulphuric acid and add strong alcohol; if boric acid be present, the alcohol will burn with a greenish flame on applying a match.

Another simple test for the presence of boric acid consists in putting about 15 ml of milk in a beaker, adding half its bulk of phenolphthalein, and dilute caustic soda solution drop by drop, with constant stirring, till a faint permanent pink colour is produced. Some of the pink-coloured milk is poured into two test-tubes. To one tube is added an equal bulk of water, and to the other an equal bulk of a neutral mixture of 1 part pure glycerol and 1 part water. In genuine milk both tubes remain pink, and the colours are practically identical, but in the presence of boric acid the water tube becomes darker in tint, and the glycerol tube much lighter—usually quite white.

Determination of boric acid

Should the presence of boric acid be detected, it may be determined by the following method, which is a modification of Thomson's process (Thomson 1896).

Seventy ml of milk are added to 7 ml of approximately 3N NaOH in a 3-in. flat-bottomed platinum dish and evaporated on the water-bath, the skin which forms being periodically removed to the side. A flame is applied to the side of the dish, care being taken that the contents do not froth over; the ignition is completed at a high temperature until the ash is almost white. The dish is cooled and 35 ml of water are added and allowed to stand until the melt is disintegrated, when it is filtered into a 100-ml flask. Five ml of approximately 3N hydrochloric acid and 15 ml of water are added to the dish, and the solution again filtered through the same filter into the same flask. The dish is further treated with 4 ml of 3N hydrochloric acid and 16 ml of water, and again filtered. To the mixed filtrates 7 ml of calcium chloride solution (10 per cent) and phenolphthalein are added, and then N NaOH is added until a slight permanent pink coloration is produced. The liquid is diluted to the mark and filtered into a graduated cylinder. Eighty ml of the filtrate are made slightly acid to methyl orange with hydrochloric acid and boiled to eliminate carbon dioxide. The solution is cooled and titrated with 0·1 N sodium hydroxide until neutral to methyl orange. Thirty ml of glycerol or 2 g of mannitol are added and the titration continued until the solution is neutral to phenolphthalein. The acidity due to 30 ml of glycerol is determined and subtracted from the number of millilitres of 0·1 N sodium hydroxide used after the addition of the glycerol; the difference multiplied by 0·011 gives the percentage of boric acid in the milk. If the amount of sodium hydroxide used exceeds 1 ml, a portion of the boric acid will be precipitated, together with the calcium phosphate. In this case the precipitate is washed with 25 ml of water through a hole made in the filter-paper; 5 ml of 3 N hydrochloric acid, a further 25 ml of water, and 0·5 ml of calcium chloride solution and phenolphthalein are added to the filtrate, and the liquid treated with N sodium hydroxide until just pink. The solution is diluted to 100 ml, filtered, and 80 ml are titrated as before. The amount of boric acid so found is added to that found in the first precipitation, after making the necessary correction for dilution.

Another modification of Thomson's process, as used in the Government Laboratory (Robertson 1923) is described under " Analysis of cream ", p. 457.

Other processes for borates

The following additional processes for the determination of borates have been described by Richmond and may sometimes be of value as confirmatory tests.

Cassal and Hehner used the following method, carried out on 25 to 50 g of sample. After addition of about 0·2 g caustic soda, the milk is evaporated and charred thoroughly by ignition; the residue is extracted by dilute acetic acid, and washed well with as small a quantity of water as possible; the solution is filtered into a small flask to which a condenser is fitted, and distilled to dryness into about 10 ml of strong ammonia; eight successive portions of 10 ml each of methyl alcohol are added and distilled off.

About 1 g of lime is ignited in a capacious platinum basin in a muffle at the highest temperature attainable, and the basin and lime weighed. The ammoniacal distillate is now added and the liquid evaporated on the water-bath; the basin is again ignited in a muffle and weighed. The increase of weight represents the boric anhydride.

Hehner preferred the use of a measured quantity of sodium phosphate solution of known strength for fixing the boric acid, instead of ammonia and lime. His method consisted in distilling directly into the sodium phosphate solution, evaporating and igniting cautiously. The weight of the residue of pyrophosphate obtained from an equal measure of sodium phosphate solution is subtracted from the weight of the residue; the difference represents boric anhydride. It is necessary, however, to ignite very cautiously, as sodium phosphate is liable to spurt.

Allen and Tankard have devised a method for the estimation of boric acid, which consists in evaporating the liquid to be tested to dryness with a few ml of 10 per cent calcium chloride solution; with milk or cream it is advisable to add just sufficient alkali solution to neutralise it to phenolphthalein.

To 10 to 25 ml add one-fifth of its bulk of 10 per cent calcium chloride solution, and just sufficient alkali to neutralise to phenolphthalein; evaporate to dryness; ignite at a gentle heat till charred thoroughly, boil the residue with 150 ml of distilled water, and filter the liquid. The filter is returned to the dish, and the residue ignited till white at a moderate temperature, and boiled with a further 150 ml of water. The liquid is allowed to stand overnight, and is filtered cold; the mixed liquids are evaporated to a volume of 25 to 30 ml, and after cooling neutralised with 0·1 N acid, using methyl orange as indicator. An equal volume of glycerol is added, and a little phenolphthalein, and the solution titrated with 0·1 N caustic soda (free from carbonate). The volume of 0·1 N caustic soda required by an equal volume of glycerol is subtracted from the amount used, and the remainder multiplied by 0·0062 will give the weight of H_3BO_3.

Richmond and Miller found that it is quite unnecessary either to evaporate the milk, ignite it, or to use any indicator other than phenolphthalein. The method is as follows. To a measured or weighed quantity of milk (10 ml suffices) add half its bulk of a 0·5 per cent solution of phenolphthalein, and run in alkali till a pink colour appears, boil, and titrate back while still boiling with acid solution till white, and finally with 0·1 N alkali till faintly pink. The colour, though faint, is quite distinct, and no attempt should be made to obtain a pronounced pink colour. Add 30 per cent of glycerol, and continue the titration with 0·1 N alkali without further heating; subtract, if necessary, the glycerol blank, when the alkali used for the final titration multiplied by 0·0062 gives the boric acid.

In place of 30 per cent of glycerol, 2 per cent of mannitol may be used or even 3 to 5 per cent of manna, as pointed out by L. E. Iles.

Cassal and Gerrans (1902) found that an intense magenta-red colour is produced on treating solutions containing boric acid with curcumin (or ordinary turmeric) and oxalic acid, and drying the mixture on the water-bath. The colour is different from that obtained by the application of the ordinary turmeric test for boric acid, and the reaction is far more delicate, extremely minute quantities of boric acid being easily detected. The colour is practically permanent for several hours—not less than ten or twelve—but fades very gradually on long keeping. The colouring-matter is readily soluble in alcohol and ether without alteration, but is destroyed by the addition of water in excess. On treatment with alkali an intense blue colour is obtained, which is different from that obtained on treating the " rose-red " colouring-matter formed in the ordinary turmeric test, with alkali. In applying the test for the detection of free or combined boric acid in milk and other food products, it is convenient as a rule to operate on the ash. The ash is treated with a few drops of (1) dilute hydrochloric acid, (2) saturated solution of oxalic acid, and (3) alcoholic solution of curcumin or turmeric, and the mixture dried on the water-bath and taken up with a little alcohol. In cases where the amount of boric acid is very small, the substance, the ash of which is to be operated upon, should be made alkaline with solution of barium hydroxide prior to evaporation and incineration. Caustic potash and caustic soda and salts of potassium and sodium in *large* amounts interfere with the formation of the colouring-matter.

Cassal and Gerrans also applied this reaction for the quantitative estimation of boric acid.

For milk, from 15 to 20 g are weighed out, transferred to a 100-ml flask, and made up to 100 ml with water. Ten ml (or more, according to circumstances) are transferred to a porcelain dish and mixed with 15 to 20 g of purified sand (obtained by igniting " silver sand ", boiling this with 25 per cent hydrochloric acid, and washing thoroughly and drying). The use of a medium such as sand is essential in order to secure intimate and complete contact between the reacting substances at the drying-point—which is the point of reaction. The mixture is made alkaline with barium hydroxide and evaporated to dryness. Two ml of a 1 per cent alcoholic solution of curcumin are added, and the mixture evaporated again to dryness, the mass being stirred from time to time to ensure thorough incorporation. To the mixture is now added 1 ml of a solution containing 25 ml hydrochloric acid and 12 g of oxalic acid in 100 ml of water, and the mass is again dried. The same operations are carried out with 10 ml of a standard solution of boric acid (1 ml being equal to 0·1 mg of boron trioxide, B_2O_3).

The colour having been obtained in both cases, the sand is extracted with ordinary alcohol.

The coloured solution obtained from the milk is diluted with alcohol or methylated spirit until the colour is of the same degree of intensity as that formed from the standard; and the amount of boric acid is arrived at by an obvious calculation.

The colours are compared in two tubes of the same internal sectional diameter (about 1 cm), placed vertically against a white porcelain plate.

On comparing the two solutions, it will be found occasionally that a certain amount of orange tint is exhibited by one or the other, due to the presence of curcumin in slight excess. When this is observed, the tints must be made the same by the cautious addition of solution of curcumin to the solution which does not show the orange tint.

The results of a number of experiments made with known amounts of boric acid and borates show that the process is reliable and accurate.

Formaldehyde

This is generally added as a 1 per cent solution in water, which is made by diluting the 40 per cent solution known as " Formalin ", " Formal ", " Formol ", or " Formine ". A very large number of reactions for this substance have been worked out. The most easily applied test is that due to Hehner, which is best carried out as follows. The milk is diluted with an equal volume of water, and a little 91 per cent sulphuric acid run in so that it forms a layer at the bottom. In the presence of formaldehyde, a violet-blue colour appears at the junction of the two liquids, and the colour is permanent for two or three days. This test will detect easily 1 part of formaldehyde in 200,000 of milk. Milk, in the absence of formaldehyde, gives a slight greenish tinge at the junction of the two liquids, and on standing a brownish colour is developed, not at the junction of the two liquids, but lower down in the acid.

Leonard and Smith's test for formaldehyde consists in heating a little milk with 3 to 5 times its volume of concentrated hydrochloric acid; a fine violet colour is produced in the presence of formaldehyde (0·0001 per cent to 0·1 per cent). The presence of a trace of ferric chloride in the hydrochloric acid is essential.

The above tests are not absolutely characteristic of formaldehyde and are not given in the presence of large amounts of this body (say 0·5 per cent). It is a reaction of the tryptophane of the casein with formaldehyde; and certain other aldehydes, e.g. vanillin, give similar colours. Acetaldehyde does not give the reaction. Leonard has pointed out that pure acids give no reaction; the presence of an oxidising agent is necessary, and he found that a trace of ferric chloride gave the best results. McLachlan (1935) finds that the present-day commercial sulphuric acid is not always satisfactory in the Hehner test, and he suggests that pure acid, to which a trace of a ferric salt has been added, should be used. The sensitivity of each delivery should be checked by means of a control test.

Hehner's test can readily be combined with the Gerber test for fat estimation by adding a trace of ferric chloride (10 ml of a 10 per cent solution of ferric chloride to 2,000 ml of Gerber acid) to the sulphuric acid used in this test. A violet ring appears at the junction of the acid and milk in the butyrometer, or a violet coloration is produced throughout the acid layer on shaking the tube, if formaldehyde is present.

Shrewsbury and Knapp's test

In the method of Shrewsbury and Knapp (1909), the reagent is made by mixing 100 ml of concentrated hydrochloric acid with 1·6 ml of N nitric acid. Ten ml of the freshly-made reagent are added to 5 ml of the milk in a test-tube, the mixture shaken vigorously and kept for 10 minutes in a water-bath at 50° C, and then rapidly cooled. As the violet colour produced varies in intensity according to the amount of formaldehyde present, the formaldehyde may be estimated by comparison with standards. If the colour obtained is deeper than that shown by 6 parts of formaldehyde per million of milk, the sample should be diluted with pure milk. The most delicate quantitative reaction is obtained with milks containing 0·2 to 6 parts of formaldehyde per million. When nitrites are present, 5 ml of the milk are treated with 0·05 g of urea and 1 ml of N sulphuric acid, and the mixture heated in the boiling water-bath for

2 minutes and then cooled; the Shrewsbury and Knapp method may then be performed in the usual way on the resulting liquid.

As a confirmatory test, some of the milk may be curdled by dilute sulphuric acid and a little Schiff's reagent—a solution of rosaniline bleached by sulphurous acid—added to the filtrate in a test-tube, which is corked and allowed to stand. In the presence of an aldehyde a violet-pink colour is produced after a short time. Excess of sulphurous acid must be avoided in preparing the reagent, or the test may fail with small amounts.

There are many confirmatory tests, which are best applied to the clear solution obtained by distilling the filtrate produced by curdling the milk with sulphuric acid. Smith and Leonard have shown that when milk containing formaldehyde is distilled, only a small fraction can be obtained in the distillate; if the milk be made alkaline, still less is obtained; but a very much larger proportion is obtained by distilling from an acid solution. This is due to the fact that formaldehyde condenses with the proteins of the milk; the more perfectly these are in a state of solution, the faster is the rate of combination. Combination is more rapid at high temperatures, but takes place at ordinary temperatures, and the total quantity added is never obtained; after a lapse of some time—several days—the formaldehyde disappears and can no longer be detected.

If Schiff's test is applied to the distillate, it must be rendered faintly acid beforehand with hydrochloric acid; Hehner has shown that the distillate of milk gives a faint pink colour with Schiff's reagent after some time, but this disappears on the addition of a drop or two of sulphurous acid, while the colour due to the presence of formaldehyde does not. He ascribes this to oxidation, but as it is equally well prevented by a little hydrochloric acid, it appears that this explanation is not correct; it is probably due to traces of alkali dissolved from the glass.

Other tests for formaldehyde

The following tests are a selection from the many which have been devised.

(1) To the distillate add one drop of a dilute aqueous solution of phenol, and pour in some strong sulphuric acid down the side of the tube. In the presence of formaldehyde a bright crimson zone appears at the junction of the two liquids. This test, which is also due to Hehner, is as delicate as the test previously described, and has the further advantage that it is given by formaldehyde solutions of all strengths. If there is more than 1 part of formaldehyde per 100,000, a white turbidity appears in the solution above the sulphuric acid, while in strong solutions a white or pinkish curdy precipitate is obtained. Hydroxy-derivatives of benzene, such as salicylic acid, resorcinol, and pyrogallol, may be substituted for phenol. Quinol, however, gives not a red colour, but an orange-yellow one. Acetaldehyde gives an orange-yellow colour with phenol and sulphuric acid.

(2) Mix the distillate with strong sulphuric acid, and sprinkle in a little morphine on the surface; a violet colour is produced in the presence of formaldehyde.

(2) To a decigram of diphenylamine add 2 ml of strong hydrochloric acid, and pour some of the distillate into the warm solution. In the presence of formaldehyde, a white turbidity or precipitate is obtained, if necessary, on further warming. The precipitate on prolonged boiling turns green. This test, like the last, is characteristic of formaldehyde, but is not of such great

delicacy as the former ones, and may not be given by milks containing only a small amount.

(4) Heat some of the milk for 30 minutes on the water-bath with a little sulphuric acid and a drop of dimethylaniline; filter; render alkaline with caustic soda; and boil until the smell of dimethylaniline has disappeared. Filter; moisten the filter-paper with acetic acid, and sprinkle lead peroxide on it. A blue colour is developed if formaldehyde is present.

(5) To the distillate add a 3 per cent solution of aniline. Formaldehyde produces a white precipitate, which is dissolved on boiling, but is deposited again on cooling.

(6) To 5 ml of the distillate add 1·5 ml of a 2 per cent solution of phenylhydrazine hydrochloride, 4 drops of ferric chloride solution, and 12 drops of sulphuric acid. A rose or dark red colour is produced in the presence of formaldehyde.

A preservative called " Mystin " was at one time used which consisted of a mixture of nitrite and formaldehyde. This mixture did not give the normal reactions for formaldehyde, but Monier-Williams found that if it was treated with urea, as described under the Shrewsbury and Knapp test, the nitrite was destroyed and the formaldehyde could then be detected and estimated in the usual way.

Determinations of formaldehyde

(1) The Shrewsbury and Knapp test can be applied quantitatively, as described above.

(2) A measured quantity of milk is acidified with a small quantity of sulphuric acid or phosphoric acid and formaldehyde determined in the distillate by Schryver's (1909) test. Ten ml of the distillate are heated for 5 minutes on a boiling water-bath with 2 ml of a freshly-prepared 1 per cent solution of phenyl-hydrazine hydrochloride. The mixture is cooled, filtered if necessary, 0·5 ml of 5 per cent potassium ferricyanide solution (or 0·5 ml of 20 volumes hydrogen peroxide) added, followed in 1 minute by 4 ml of concentrated hydrochloric aicd. After standing 10 minutes, if formaldehyde is present a red coloration is obtained, which is matched in depth against measured quantities of standard formaldehyde solution treated similarly.

Salicylic acid and benzoic acid

Revis's method (1912)

For the detection of benzoic and salicylic acids, the milk or cream is made alkaline with sodium carbonate and the casein precipitated by heating on a water-bath with $\frac{1}{10}$ the volume of 10 per cent calcium chloride solution; after cooling, the filtrate is neutralised and the proteins removed by treatment with copper sulphate and sodium hydroxide. The filtrate from this is acidified and extracted with a mixture of ether and petroleum ether, the solvent washed with water, and finally a small quantity of water and a drop or two of phenolphthalein added, and dilute caustic soda dropped in with constant shaking till the aqueous portion is pink. This should be removed, just acidified with very dilute acetic acid, boiled to expel ether, and to a portion a drop of dilute ferric chloride solution is added: a violet coloration is developed in the presence of salicylic acid, while benzoates give a buff precipitate, insoluble in dilute acetic acid. To confirm the presence of salicylic acid, a portion is tested with bromine water: a curdy yellowish precipitate is produced by salicylic acid, and the characteristic smell of halogen-phenol derivatives is developed; another portion is evaporated to dryness with strong nitric acid, and the residue taken up with a few drops of water; a yellow coloration is produced on adding ammonia if salicylic acid be present.

Hinks's method (1913)

Ten to 20 g are treated with an equal volume of concentrated hydrochloric acid till the curd is dissolved, and, after cooling, shaken with 25 ml of 1 part of ether to 2 parts petroleum ether. The ethereal layer is separated and 1 drop of 0·880 ammonia added; if benzoic acid is present a precipitate occurs in the ethereal layer.

Five ml of water are now added, the mixture is shaken well, the water separated and heated on the water-bath to expel ammonia, and tested as above for salicylic and benzoic acids. If the extraction is repeated twice more, and the ethereal extracts washed three times with about 5 ml of water, the salicylic acid and benzoic acids can be titrated by adding 5 ml of water, a little phenolphthalein, and the 0·1 N alkali till the water is just permanently pink. The titration may be checked by evaporating and weighing the alkaline salts.

These reactions are not absolutely characteristic of salicylic acid, as phenol (carbolic acid) and other hydroxy-benzene derivatives behave in a similar manner, but Self's test appears to be characteristic. A little of the dry residue is moistened with a cold mixture of equal parts of strong sulphuric acid and 40 per cent formaldehyde, and a little ammonium vanadate added on the end of a glass rod. A Prussian-blue colour indicates salicylic acid.

Jorissen's test consists in adding to the solution 5 drops of a 10 per cent solution of sodium nitrite, 5 drops of 50 per cent acetic acid, and one drop of a 1 per cent solution of copper sulphate. The solution is heated in a water-bath for 45 minutes, when salicylic acid gives a red colour.

Salicylic acid may be estimated by comparing the colour given with ferric chloride with that given by known weights of salicylic acid.

Biernath destroys salicylic acid by heating the solution with alkaline permanganate; at the Government Laboratory 1 ml of permanganate solution (2 g $KMnO_4$ and 4 g KOH in 100 ml) is added to the solution, which is then heated on the water-bath for 15 minutes; if the colour is discharged, further additions of permanganate are added till the colour is permanent. The excess is removed, after acidifying with sulphuric acid, by oxalic acid, and the solution can then be distilled or extracted with a solvent to separate benzoic acid, if present.

Benzoic acid gives the reactions below. If salicylic acid is present, a little bromine water is added, and a turbidity or precipitate will be produced; addition of bromine water should be continued till all the salicylic acid is precipitated, and the precipitate removed by filtration. The excess of bromine should be boiled off, and the following tests applied—

(*a*) Add a few pieces of magnesium and hydrochloric acid till gas begins to be evolved; benzoates are reduced to benzaldehyde, which has a characteristic smell.

(*b*) Evaporate a little of the solution with soda-lime, and ignite in a current of inert gas (nitrogen formed by passing air through alkaline pyrogallol serves);

benzoates are reduced to benzene (characteristic smell), which may be collected in a mixture of nitric and sulphuric acids, which form nitrobenzene (another characteristic smell); this may be converted into aniline, diazotised and condensed with β-naphthol (red colour).

(*c*) Evaporate a little of the solution to dryness, add 2 ml of aniline and 0·02 g rosaniline hydrochloride, and boil for twenty minutes; a blue colour is produced if benzoates are present.

(*d*) Evaporate a little of the solution to dryness, add a little gallic acid and 1 ml sulphuric acid; if benzoates are present anthragallol is produced, which, on dilution and making alkaline, gives a red colour, passing to brown.

(*e*) Jorissen tests for benzoic acid by oxidising it to salicylic acid with hydrogen peroxide. To the solution to be tested (about 25 ml) 2 drops of 1 per cent ferric chloride solution are added and, if salicylic acid be absent, 2 drops of a dilute solution of hydrogen peroxide (one volume strength). On standing, the violet colour due to salicylic acid gradually appears.

Illing (1932) has published an important paper on the separation of benzoic acid from foodstuffs, including milk, and its ultimate determination by *Mohler's test*, i.e. by comparison of the red coloration due to *m*-diamino-benzoic acid, which is formed in the test. Separation of benzoic acid is effected by distilling a portion of the foodstuff containing from 3 to 7 mg of benzoic acid in a flask containing in addition 5 ml of 10 per cent sulphuric acid, 40 gm of salt, a little powdered pumice, and enough water to make the volume up to 120 ml. The distillate is led by means of a still-head into an ordinary calcium chloride tube, which contains 1·5 ml of N sodium hydroxide solution and sufficient water to form a seal. The other limb of the calcium chloride tube contains a vertical glass tube, 16 inches long. During the experiment the calcium chloride tube is immersed in a beaker of boiling water. After distilling for 12 to 15 minutes, the contents of the calcium chloride tube are evaporated and benzoic acid is determined by a modification of Mohler's test, which must be carried out strictly as follows—

The benzoic acid residue (as the sodium salt[1]) is introduced into a boiling-tube (6 × $\frac{3}{4}$ in.), and the tube is heated in a beaker of boiling brine until the liquid is driven off, and all the drops of condensation water have disappeared. It is cooled, and 0·1 g of potassium nitrate and 1 ml of concentrated sulphuric acid are added. The tube is placed in boiling water for 20 minutes, then cooled, and 2 ml of water are added. The tube is held under running water, and 10 ml of 15 per cent ammonia solution are carefully added, followed by 2 ml of a 2 per cent hydroxylamine hydrochloride solution, and the contents are well mixed. It is next placed in a beaker of water at 65° C for 5 to 6 minutes, then cooled, and the colour matched with that developed by mixing the amounts of iron-ammonium-alum and potassium thiocyanate solutions given in Table 18.1 for varying quantities of benzoic acid.

The standards of 15 ml volume are made up in a graduated cylinder by adding the requisite amounts of the two constituents and making up to 15 ml, which is the volume of the solution obtained at the completion of the test. The colours are matched in 50-ml Nessler tubes. For 6 mg or more of benzoic acid it is better to dilute to 50 ml and to use 100-ml Nessler tubes. These standards cannot be diluted, as the colours are not proportional in other dilutions.

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[1] Ammonium benzoate is volatile at 100° C.

Table 18.1

Benzoic acid mg	Iron-ammonium-alum solution (1 g per litre) ml	Potassium thiocyanate solution (2 per cent) ml	Volume made up to ml
0·1	0·2	0·2	15
0·25	0·3	0·3	15
0·5	0·5	0·5	15
0·75	0·7	0·6	15
1·0	1·0	0·8	15
2·0	1·5	1·5	15
3·0	2·0	1·5	15
4·0	2·0	2·3	15
5·0	3·0	2·5	15
6·0	4·0	3·5	50
7·0	4·0	4·5	50
8·0	4·5	3·5	50
9·0	5·0	4·0	50
10·0	5·5	5·5	50

The above test will detect 0·025 mg of benzoic acid, and amounts of this acid differing by 0·025 mg can be distinguished when working with 0·1 mg or less, but with increasing depth of colour in more concentrated solutions of benzoic acid the sensitiveness of the test decreases.

Salicylic acid, phenolphthalein, saccharin and vanillin all give yellow colours only. Cinnamic acid gives a brownish colour. Fatty acids give no colour at all.

Monier-Williams (1927) suggests a method for the determination of benzoic acid in non-fatty foodstuffs, depending on steam distillation of benzoic acid from acidified brine. After evaporation and purification, the acidified distillate is extracted with a mixture of methylated ether and petroleum ether. The solvent is evaporated and the benzoic acid sublimed at 160° C and weighed. While this process is only recommended for non-fatty foodstuffs, sublimation could be used as a method of purification and for determination of the benzoic acid obtained by extraction from milk.

Liverseege and Evers (1913) suggested steam distillation as a means of determining benzoic acid in milk. One hundred ml of the sample are acidified with 10 ml of strong sulphuric acid and steam-distilled until 600 ml of distillate are obtained. This is then acidified, extracted with ether, the ether evaporated and the residue weighed. The method does not, however, give anything like a theoretical yield, as approximately only 45 per cent of the benzoic acid present in the milk is recovered.

β-naphthol

This is best detected by taking advantage of its easy condensation with tetrazonium salts in faintly acid solution to form dark red compounds.

Richmond and Miller tested as follows. To 1 gm of benzidine add 4 ml strong HCl and about 60 or 70 ml of water; keep this solution cool, and add little by little 1 g sodium nitrite dissolved in about 25 ml of water, cooling and shaking well between each addition. When all the nitrite has been added, nearly neutralise, using phenolphthalein as indicator. To a few ml of milk add a little of this solution; if β-naphthol is present, a red colour will be produced. *Do not make alkaline*, as milk itself gives a brownish-red colour in alkaline solution.

As a confirmatory test, a diazotised solution of phenyl-hydrazine may be used, which gives a red colour in alkaline solution with β-naphthol, but no colour with milk.

If the milk is extracted with chloroform, and the chloroform heated with caustic potash for a few minutes, a deep blue colour indicates the presence of β-naphthol.

Fluorides

Fluorides are detected in the ash of milk. At least 25 ml of milk should be taken, and the ash treated in a platinum basin with a little strong sulphuric acid. Over the top of the basin a watch-glass coated with beeswax, through which a few lines are scratched, is placed, and a piece of ice or some cold water is put into the concave depression. The basin is then warmed gently and the watch-glass exposed to the action of the fumes evolved for ten minutes. In the presence of fluorides it is seen that the glass has been etched, after removal of the wax. If a drop of water is placed on the wax, away from the lines scratched through it, a white film of silica will be formed on its surface if *fluosilicates* be present. If *fluoborates* be present, this drop of water will give a boric acid reaction; in the presence of fluoborates both a fluoride and a boric acid reaction are given by the ash of the milk.

O. and C. Hehner pointed out that when there is much boric acid in relation to the fluoride present, the test for fluorides applied directly to the ash fails. The milk should be made alkaline, ashed, and the ash dissolved in a little acid; calcium chloride is added, and the solution made alkaline with ammonia; the precipitate is collected, burnt, and extracted with acetic acid, and the test made on the insoluble portion.

A modification of the Monier-Williams test for fluorides in butter may be used for milk. To a few ml of the milk are added a few drops of hydrogen peroxide and 1 ml of a solution containing 2 per cent titanium sulphate in 10 per cent sulphuric acid. A blank on pure milk is carried out at the same time. In the presence of fluorides, the orange-yellow colour of the peroxide will be partially or wholly discharged. While not specific, it is a useful sorting test, the results of which may be confirmed by testing the ash as above.

Determination of fluorides (*S.P.A. method*) (1944)

The distillation apparatus consists of a Claisen flask of 100–150 ml capacity, a large flask for generating steam and an efficient condenser. The main neck of the Claisen flask is fitted with a two-holed rubber stopper through which pass a thermometer and a glass tube for connecting with the steam supply, both the thermometer and the tube extending almost to the bottom of the flask. The side neck of the Claisen flask is closed with a solid rubber stopper and the side arm connected with the condenser. The steam is generated from water made alkaline with sodium hydroxide. Local overheating of the Claisen flask is avoided by use of an asbestos board with a hole which must fit closely to the lower surface of the flask or by use of an asbestos gauze.

Mix a suitable quantity of the sample (10 g or less according to the amount of fluorine expected) in a platinum dish with about 1 g of fluorine-free lime and 50 ml of water, evaporate on a water-bath and thoroughly char at a temperature below visible red heat. Transfer to a muffle at about 600° C (dull red heat) and ignite for 1½ to 2 hours.

Assemble the apparatus, introduce into the flask a number of fragments of Pyrex glass, 0·2 g silver sulphate or sufficient to precipitate all the chloride in the portion of sample being treated, 7 ml of water and 15 ml of 60 per cent perchloric acid. Heat the flask until the temperature reaches 120°–125° C, connect the steam supply, regulate the gas and steam supplies so that the temperature of the distillation is maintained at 137°–140° C and distil 150 ml in 25–35 minutes, steaming out the condenser towards the end of the distillation. Discard the distillate. Distil a further 150 ml and titrate an aliquot part by the method given below. Calculate the amount of fluorine in the whole fraction. (This figure, which should not exceed 1·5 mg, may be termed the "apparatus blank" and should be approximately constant for any further 150 ml fractions.)

Cool the flask, transfer the acid contents to a suitable receptacle and rinse the flask and glass fragments with distilled water, rejecting the rinsings. Introduce the bulk of the dry ash into the flask and wash in the remainder with about 5 ml of water containing a few drops of the acid. Add the remainder of the acid, whilst cooling the flask, and rinse down the neck of the flask with 1–5 ml of water. Connect up the apparatus and distil 150 ml as before.

Titrate 50 ml of the well-mixed distillate with 0·05 N sodium hydroxide in a Nessler tube, using methyl orange as indicator, until the colour matches that of a comparator tube containing distilled water and the same amount of methyl orange.

Transfer the remaining 100 ml of distillate to a Nessler cylinder and add an amount of 0·05 N hydrochloric acid to make the total acidity equal to 5·0 ml of 0·05 N acid. Prepare a "control" cylinder containing 5·0 ml of 0·05 N hydrochloric acid and distilled water and add to both "test" and "control" cylinders 2 ml of 0·01 per cent alizarin S solution. From a burette which can be read to 0·02 ml add to the "test" cylinder a solution of thorium nitrate (approximately 0·25 g per litre) until a slight pink colour persists as compared with the yellow of the "control" cylinder. Add an exactly similar volume of the thorium nitrate solution to the "control" cylinder, which then becomes more pink than the "test" solution. Then add slowly, from a suitable burette, standard solution of sodium fluoride (0·0221 g of NaF per litre; 1 ml = 0·01 mg of fluorine) until the tints of "test" and "control" solutions match exactly. The volume of standard fluoride solution added corresponds to the amount present in the "test" portion of the distillate. Calculate the amount of fluorine present in the 150 ml of distillate and subtract the "apparatus blank". Express the result as parts per million of the food.

The fluorine-free lime may be prepared in the following manner.

> Prepare an ammonium carbonate reagent by dissolving 110 g of ammonium carbonate of Analytical Reagent purity, and 55 ml of ammonia (sp. gr. 0·880) in water and diluting to 600 ml.

Dissolve 200 g of calcium chloride (dry) of A.R. purity in about 600 ml of warm distilled water. Stir into this solution 20 ml of the ammonium carbonate reagent, bring the mixture just to boiling point, allow the precipitate to settle for a few minutes and then filter on a Buchner funnel with suction and reject the precipitate. Repeat the precipitation and rejection three times with 20 ml portions of the ammonium carbonate reagent. Finally treat the clear filtrate from the last precipitation with the remainder of the ammonium carbonate reagent, stir the mixture well and bring it to the boiling point. Allow the precipitate to settle, filter, wash several times with hot water until free from chloride and dry at 100° C. Ignite to oxide in a platinum dish in small quantities of 1 g to 2 g as required.

An addendum (1945b) to this method recommends a modification if the amount of phosphate in the material is such that more than 0·5 g of P_2O_5 is present in the distillation flask, since traces of phosphoric acid might come over in the distillate and be calculated as fluorine. In such cases two successive 150 ml portions of distillate should be collected. If the second portion shows any appreciable fluorine content the first portion should be evaporated to dryness with 1 g of fluorine-free lime and re-distilled to give 120 ml of distillate. Results calculated from the titration of the distillate give the true fluorine content of the sample.

Hydrogen peroxide

This substance has been employed as a preserving agent for milk. Budde patented a process which consisted in adding hydrogen peroxide to milk, and heating to 50° to 55° C to complete the liberation of oxygen by the catalase of the milk. It appeared to act by liberating oxygen in the interior of the micro-organisms present and thus bursting them. The peroxidase of the milk is also destroyed by excess of hydrogen peroxide. It therefore follows that if a milk contains abundance of soluble albumin and yet does not give the paraphenylene-diamine or " ortol " reactions (p. 415), it is probable that it has been treated by the above process.

Hydrogen peroxide may be detected in milk by adding to a small quantity of fresh milk a little orthomethyl-amino-phenol sulphate or some " ortol " (i.e. a mixture of this body with quinol, used in photography) and adding an equal bulk of the suspected milk. In the presence of hydrogen peroxide a red colour will be produced.

Werther's test consists in adding 10 drops of a 1 per cent solution of sodium orthovanadate in 10 per cent sulphuric acid to 10 ml of milk; hydrogen peroxide gives a distinct red coloration. Titanium sulphate solution, as used in the Monier-Williams test for fluorides, will also give an orange-red coloration in the presence of hydrogen peroxide.

In the presence of hydrogen peroxide a blue coloration is obtained on the addition to milk of potassium iodide and starch solution.

Hinks (1915) investigated the persistence of hydrogen peroxide in milk, the catalytic activity and the peroxidase reactions of milk. From the results of his experiments it is improbable that amounts of less than 0·1 per cent of hydrogen peroxide, added to perfectly fresh milk and maintained at room temperature, would persist for more than 24 hours. On the other hand, if large amounts (0·2 per cent or more) are added to milk under the same conditions, a considerable proportion will remain after several months, or even a year.

When hydrogen peroxide is added to stale milk, it does not persist for anything like the same length of time.

Hinks used for the determination of hydrogen peroxide in milk Konnig's iodometric method as modified by Revis (1910). Place 5 ml of the sample in a 250 ml stoppered flask, add 10 ml of concentrated HCl, shake, then add 10 ml of 10 per cent potassium iodide and after 15 minutes 100 ml of water. The liberated iodine is titrated with 0·1 N thiosulphate, with starch paste as indicator. Owing to the occlusion of iodine by the casein, the titration takes about 30 minutes to complete.

Nitrites

If the Gerber method of fat testing is used, an indication of the presence of nitrites or nitrates will be obtained by the fact that a golden-brown colour is produced on shaking the butyrometer (Elsdon and Sutcliffe 1913). This coloration is quite distinct from the gradual production of the purplish-brown coloration obtained with pure milk.

The confirmation of the presence of nitrates is dealt with on pp. 408 to 410.

There are two methods in general use for the determination of nitrites in small quantities, namely the metaphenylene-diamine (see below) and the Griess-Ilosvay methods. Of these the latter is by far the more delicate, and the colour produced is easier to match. The reagent is made up as follows: 0·1 g of α-naphthylamine is dissolved in 20 ml of glacial acetic acid, and mixed with 0·5 g of sulphanilic acid dissolved in 150 ml of warm 6 per cent acetic acid, the whole being diluted to 300 ml with more 6 per cent acid; 150 ml of 60 per cent acetic acid are added to this. In carrying out the test, 5 ml of the milk are placed in a test-tube (4 × ½ in.) and 3 ml of the reagent are added. Milks containing known quantities of nitrite are treated in the same way. The colour produced is matched with the standards after about 1 hour.

The metaphenylene-diamine or Griess test is carried out by adding 1 ml of a 0·5 per cent solution of metaphenylene-diamine in very dilute sulphuric acid and 1 ml of sulphuric acid (33 per cent) to a portion of the sample; a brown coloration after 20 minutes indicates nitrites. As previously stated, this test is not as delicate as the Griess-Ilosvay test, but it may also be employed for the determination, by the use of a series of standards containing varying amounts of a standard nitrite solution (1 ml ≡ 0·00001 g nitrogen). It is important in both these tests that the reagents should be added to the sample and standards at the same time, as the colours deepen on standing.

Chlorine

The use of oxidising or preservative substances in the cleansing of milk utensils is forbidden, except under certain conditions in the case of mechanical milkers, in this country. There is always the possibility, however, that oxidising agents may be used for this purpose, or even as a preservative in the milk itself. Hypochlorites are the most likely substances to be used for cleansing and sterilising purposes, and tests, therefore, usually resolve themselves into tests for free chlorine. Hale and Bleecher (1924) state that the ordinary *o*-toluidine test for free chlorine is not applicable to milk and gives no colour with quite high concentrations.

Wright and Anderson (1938) have, however, developed a test as the result of investigating some work done on this subject by Rupp (1922). The details of the test are as follows. To 3 ml of milk in a $\frac{3}{4}$-in. test-tube, cooled to 0° to 5° C, are added 3 ml of 73·5 per cent sulphuric acid containing 0·025 per cent of stannous chloride, also cooled to 0° to 5° C. The contents of the tube are well shaken in a freezing mixture of ice and salt, and allowed to stand in this mixture for 3 minutes. The contents are then transferred to a 12·5 ml centrifuge tube and spun for 3 minutes at 2,500 revolutions per minute. The tubes are immediately examined in ultra-violet light for the presence of any yellow fluorescence. The test is not due directly to chlorine but to chlorate, which is present in all hypochlorite solutions. Approved hypochlorite solutions for use on farms and in dairies must contain 0·7 per cent sodium chlorate. The test can be carried out 4 days after the addition of hypochlorite without appreciable loss in sensitivity, and will detect an addition of hypochlorite equivalent to 13 parts per million of chlorine. By taste it is not possible to detect less than 50 to 100 parts per million of available chlorine in milk.

p-hydroxybenzoates

Various esters of *p*-hydroxybenzoic acid are used as food preservatives in the United States and other countries, although their use is forbidden in Great Britain. The most common of these are the methyl, ethyl, and propyl esters.

Edwards *et al.* (1937) have published a method for the detection and estimation of *p*-hydroxybenzoates in foodstuffs, and the following is their modification as applied to milk.

Twenty-five ml of milk are pipetted into a 50-ml graduated flask and the fat and proteins precipitated by the addition of 5 ml each of zinc acetate solution (21·9g $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ and 3 ml glacial acetic acid in 100 ml) and potassium ferrocyanide solution (10·6 g $K_4Fe(CN)_6 \cdot 3H_2O$ in 100 ml). After making up to 50 ml with water, the contents of the flask are well mixed, allowed to stand for 5 minutes and filtered. Thirty ml of the filtrate are saturated with ammonium sulphate, extracted with three 15-ml portions of ether, the extracts combined in a flask, and the solvent distilled off. To the residue are added 60 ml alcohol and 5 ml 0·5 N alcoholic potash and the mixture boiled for two hours under reflux. After removing the bulk of the alcohol by evaporation, the residue is diluted with water, acidified with dilute sulphuric acid, saturated with ammonium sulphate, and extracted with three 15-ml portions of ether. The mixed ethereal extracts are extracted with three 10-ml portions of dilute ammonia, the combined ammoniacal extracts boiled until the solution is neutral to litmus, cooled, and made up to a suitable volume.

For the detection of the acid the production of a rose-red colour with Millon's reagent may be used, but an orange-red colour is given by salicylic acid so that an alternative test must be used if this latter is present. Edwards *et al.* recommend the formation of the nearly insoluble copper salt as a suitable qualitative test. To a suitable volume of the solution of the neutral ammonium salt (containing from 2 to 5 mg of the acid) is added 1 ml of 2 per cent copper sulphate solution. After evaporation to a volume of about 1 ml on the water-bath it is allowed to cool, when characteristic crystals are formed. This reaction is not given by benzoic or salicylic acid.

The acid is estimated in the following manner.

Twenty ml of the neutral ammonium salt solution (containing not more than 2 mg of the acid) and 2 ml freshly-prepared Millon's reagent in a boiling-tube are heated in a boiling water-bath for 2 minutes, immediately diluted to 50 ml in a Nessler tube, and the colour compared with that of standards prepared at the same time and in the same way. A suitable series consists of 1, 2·5, 5·0, 7·5 ml, etc., of an aqueous solution of *p*-hydroxybenzoic acid (1 ml $\equiv$ 0·1 mg) diluted to 20 ml.

By this process the authors of the method obtained about 60 per cent recovery of the ethyl ester, although recovery of various esters from other foodstuffs was of the order of 80 to 90 per cent. When salicylic acid is present it can be estimated by the ferric chloride method and the appropriate amount added to the *p*-hydroxybenzoic acid standards in order to make allowance for its production of colour with the Millon's reagent.

(2) DETECTION OF NITRATES IN MILK

Although potassium nitrate is only a comparatively weak antiseptic, its use as a preservative or deodoriser in milk has been reported (Elsdon and Sutcliffe 1913, Elsdon and Smith 1922, Wood 1932). It is, however, as an indirect means of proving the presence of extraneous water that the testing of milk for nitrates has proved most valuable. In many districts the water supply contains appreciable quantities of nitrates in solution, and should such water be used for the adulteration of milk, the latter yields a positive reaction for nitrates. There are no authenticated cases of nitrates being found in pure cows' milk, although Richmond (1893, 1894) has reported instances of its presence. Richmond himself later (1934) cast doubt upon his own results, and Elvidge (1934) points out that results apparently positive may be due to the test generally used not being specific, and that it would be given by hypochlorites, which might gain access to the milk as the result of their being used to cleanse mechanical milkers. Although there is no definite evidence of the presence of nitrates in pure cows' milk, there appears, however, to be little doubt that nitrates are found in human milk (Lerrigo 1930).

In so far as cows' milk is concerned, in districts where the water supply contains nitrates, the detection of nitrates is a valuable confirmatory test, which must rank next to the freezing-point test as a means of differentiating between milks naturally deficient in solids-not-fat and those in which the deficiency has been brought about by the addition of extraneous water.

Elsdon and Sutcliffe (1913) point out that when the Gerber method is used for fat estimation, a golden-brown coloration will be produced on shaking the butyrometer when either nitrates or nitrites are present, and this colour is quite distinct from the brownish-purple colour produced by genuine milks. The method of testing usually adopted depends on the formation of a blue coloration by a solution of diphenylamine sulphate in concentrated sulphuric acid in the presence of either nitrates or nitrites. It follows, therefore, that the sample should also be examined for nitrites by the Griess-Ilosvay test, and if this is positive, nitrates should be tested for by the brucine–sulphuric acid reagent (see below). Milk to which nitrates have been added usually also gives a reaction for nitrites after two days, and the quantity then slowly increases to a maximum and then decreases. The following table illustrates this point—

Table 18.2—Development of nitrite in milk containing nitrate (*Elsdon and Sutcliffe*)

	Parts per 10^6 nitrite found				
	1st day	2nd day	3rd day	4th day	5th day
Milk containing 100 per 10^6 KNO_3	0	2	8	25	12
Milk containing 20 per 10^6 KNO_3	0	0·2	2	4	0

The diphenylamine test

Elsdon and Sutcliffe's method of carrying out the diphenylamine test is as follows—

Preparation of milk serum. The method is a modification of that due to Tillmans and Sutthoff (1910). The reagent is a 2·5 per cent solution of mercuric chloride in 1 per cent wt/vol hydrochloric acid. Twenty-five ml of milk are mixed with 25 ml of the reagent, and after well mixing, the whole is poured on to an 11 cm filter-paper and 25 ml of filtrate collected.

The test is also a modification of the method of Tillmans (1911). The reagent is prepared by adding 50 ml of water to 0·085 g of diphenylamine sulphate contained in a large flask, and gradually adding 450 ml of strong sulphuric acid. In performing the test, 1 ml of the serum of the suspected milk is placed in a 4 × ½ in. test-tube, 4 ml of the reagent added, and the whole thoroughly mixed and cooled. The presence of traces of nitrates causes the production of a blue colour.

Lerrigo (1930) has modified the method of application of the diphenylamine test to make it more suitable for routine purposes; his procedure is as follows—

(1) *Mercury reagent.* This consists of an aqueous solution containing 20 per cent of mercuric chloride, 5 per cent of ammonium chloride, and 20 per cent (by volume) of hydrochloric acid. (The presence of ammonium chloride is necessary to keep the mercuric chloride in solution.)

(2) *Diphenylamine reagent.* (As above.)

METHOD—Six or seven drops of the mercury reagent are added to 4 or 5 ml of milk in a test-tube (previously washed with some of the milk), the tube shaken occasionally during two minutes, and the mixture filtered through a 9 cm paper (previously washed with distilled water) into a test-tube (6 × ½ in.) containing about 2 ml of the diphenylamine reagent. (This should be introduced into the test-tube by means of a pipette, i.e. without unduly wetting the sides with the reagent.)

The filtration should be conducted with the test-tube containing the diphenylamine in an oblique position, so that the filtered milk serum, which is usually quite clear, falls on to the side of the tube and flows on to the surface of the reagent fairly gently, so as not to mix with it to any great extent. When about 1 ml of the serum has been collected, the funnel is removed and the test-tube is held vertically and examined against a white glazed surface.

With pure milk, no colour or only a yellow to brown coloration is produced. If the milk contains large amounts of nitrate, the surface of contact between serum and acid becomes dark blue, and the colour spreads upwards on shaking. With smaller quantities (i.e. of the order of 0·1 part of nitric nitrogen per 100,000 or less) there is no colour until the tube is gently agitated, when the blue colour appears at the bottom of the serum, and below this again appears the yellow ring produced by normal milk.

The reagents and test-tubes used in the test should be kept in a room in which strong nitric acid is neither used nor kept. Blanks on genuine milk should always be carried out alongside the actual sample, and any weak positive results should always be repeated.

Lerrigo claimed that his test will detect the addition to milk of 5 per cent of a water containing about 0·5 part of nitric nitrogen per 100,000.

Monier-Williams (1931) recommends that diphenylbenzidine should be used instead of diphenylamine for the nitrate test. Diphenylbenzidine is the intermediate product formed in the reaction, and its use renders the test approximately ten times more sensitive. In other respects the method of carrying out the test is identical with the diphenylamine test. In the same paper Monier-Williams has given full details of Marqueyrol and Muraour's (1914) method for the preparation of diphenylbenzidine.

The brucine–sulphuric acid method

Elsdon and Sutcliffe use this method when both nitrates and nitrites are present.

Reagent. This is prepared by dissolving 0·2 g of pure brucine in 75 ml of sulphuric acid (sp. gr. 1·82) and adding this to 25 ml of water.

In the absence of nitrites, 5 ml of the serum are placed in a test-tube and 10 ml of the reagent are added. The contents are well mixed, poured into a Nessler cylinder and allowed to stand for half-an-hour. The colour is then compared with standards prepared in the same manner.

In the presence of nitrites, the colour is produced immediately on the addition of the reagent, and then quickly commences to fade owing to the bleaching action of the nitrite. This action is practically independent of the amount of nitrite present—at least, for quantities between 2 and 40 parts per 100,000. The procedure adopted is therefore as follows. Five ml of the serum are placed in a test-tube, and 10 ml of the reagent are added. At the same time, standards are prepared containing suitable quantities of nitrate, and each containing about 10 parts per 100,000 of sodium nitrite. The contents of the tubes are poured into Nessler cylinders, and matched any time after three hours.

(3) DETECTION OF ADDED COLOURING-MATTER IN MILK

The addition of any kind of colouring-matter to milk is prohibited in Great Britain. It can generally be assumed that colouring-matter is added to milk to conceal the fact that it has previously been adulterated by skimming or by the addition of water. It is, however, an offence to add colouring-matter to milk which is not otherwise adulterated.

If a sample of milk is very yellow in colour it should be examined for added colouring-matter, but it by no means follows that this will be present, e.g. the milk yielded by Jersey cows and by cattle fed on Cheshire pastures is often naturally deep-yellow in colour. *Annatto* is still the colouring-matter which is chiefly used for the purpose of adulteration. Coal-tar colours, especially the sodium salt of dimethyl-amino-azo-benzene-sulphonic acid or methyl orange, are sometimes used, and occasionally phosphine (diamino-phenyl-acridine, usually mixed with diamino-toluyl-acridine), caramel, saffron, carotene, or turmeric have been suggested.

Annatto is a reddish-yellow colouring-matter derived from the pulp of the fruit of *Bixa orellana*, a shrub native to South America and the West Indies.

The following general method for the examination of milk for colouring-matter is due to Leach (1920) and is similar to the official method of the A.O.A.C. (1950). About 150 ml of the sample are curdled by the aid of heat and acetic acid, preferably in a porcelain dish over a bunsen flame. By the aid of a stirring-rod the curd can usually be collected into a compact mass, and the whey is then simply poured off. Should the curd, however, remain floating, separation can be effected by means of a sieve. The whole of the annatto or coal-tar dye and part of the caramel, if present in the milk treated, will be found in the curd. The curd, pressed free from adhering liquid and picked apart if necessary, is shaken with ether in a corked flask, in which it is allowed to soak for several hours, or until the fat has been extracted and with it the annatto. If the milk is uncoloured, or has been coloured with annatto, on pouring off the ether the curd should be left perfectly white. If aniline dyes or caramel have been used, the curd will be coloured. In other words, annatto is the only added colour extracted by ether.

Tests for annatto

The ether, after separation of the curd, is evaporated and the residue made alkaline with a little dilute solution of sodium hydroxide and poured upon a small wet filter-paper. The fat is thereby held back, and the aqueous filtrate as it passes through allows the annatto, if present, to permeate the pores of the filter. On washing off the fat by means of a gentle stream of water from the tap, the annatto will be found as an orange-brown stain on the paper. Stannous chloride solution applied to the coloured filter produces a characteristic pink colour.

Tests for caramel

The two following tests are given by Leach (1920). If the fat-free curd is coloured after the ether has been poured off, it is examined further for caramel or coal-tar dyes. A portion of the curd is placed in a test-tube and shaken vigorously with concentrated hydrochloric acid. If caramel is present, the acid solution of the coloured curd will gradually turn a deep blue on shaking, as would also the white fat-free curd of an uncoloured milk, the blue coloration being formed in a few minutes if the fat has been thoroughly extracted from the curd. The curd must be fat-free for the test to be effective, and gentle heat will hasten the production of the blue colour. It is only when a blue colour of the acid occurs in conjunction with a coloured curd that caramel is to be suspected. If much caramel be present the acid solution may be brownish-blue. If the above test is positive it should be confirmed as follows—

Approximately 150 ml of the sample are curdled with an equal volume of strong alcohol. The whey is filtered off and a small quantity of lead subacetate is added to it. The precipitate produced by this latter reagent is filtered upon a small filter and dried in a place free from sulphuretted hydrogen. Pure milk yields a residue which is not more than pale straw colour, whereas in the presence of caramel the residue is more or less dark-brown in colour, depending on the amount of caramel used.

Test for coal-tar colours

If the milk has been coloured with an azo dye, a portion of the coloured curd, treated with strong hydrochloric acid in a test-tube, will immediately turn pink. This reaction is more delicate than applying hydrochloric acid to the milk itself.

Preliminary tests

The following tests are sometimes used to indicate coloured samples, but they are not of course as delicate as the general method of testing described above.

(1) Allow a portion of the milk to stand in a cool place until the cream rises; if the skim-milk is more highly coloured than the cream, the milk is artificially coloured.

(2) Add a drop or two of hydrochloric acid to a little milk; a pink colour indicates the presence of an azo colour, of which the following, among others, may occur in milk—

Aniline yellow. Amino-azo-benzene.
Butter yellow. Chrysoidine. Dimethyl-amino-azo-benzene.
Acid yellow. Salts of amino-azo-benzene-sulphonic acid.
Methyl orange. Salts of dimethyl-amino-azo-benzene-sulphonic acid.
Orange IV. Diphenylamine yellow. Salts of diphenylamine-azo-benzene-sulphonic acid.

(3) Make the milk alkaline with sodium bicarbonate, and immerse a strip of filter-paper therein for at least twelve hours. A reddish-yellow stain, changed to pink by stannous chloride, indicates annatto.

These tests may fail to show artificial colouring-matters, because (1) aniline yellow and butter yellow are soluble in fat, and may rise with the cream; (2) azo compounds are reduced in stale milk to colourless compounds; and (3) a colour such as phosphine (diamino-phenyl-acridine, usually mixed with diamino-toluyl-acridine) or caramel has been used.

Gardiner (1925) found that annatto could still be detected in milk after keeping for 15 months. By the following modification of Leach's method and working upon 25 ml of sample, he could still detect as little as 0·03 grain per gallon. Twenty-five ml of milk are coagulated in a 100-ml conical flask by warming to 50° C and adding 0·2 ml of glacial acetic acid. The whole is then poured into a Buchner funnel (3 in. diameter) in which there is fitted a No. 41 Whatman filter-paper. After pressing the curd to free it from water, it is returned to the original conical flask, shaken vigorously for a minute or so (to disintegrate the curd) with 75 ml of ether, and allowed to stand overnight. The ethereal solution, which contains the fat and any annatto present in the milk, is poured into a porcelain basin and evaporated to dryness on a water-bath. Immediately it reaches this stage it is made alkaline with 6 ml of 0·1 N sodium hydroxide solution, stirred thoroughly, and transferred to a wet 9-cm filter-paper. When all the liquid has passed through and only fat remains, the filter-paper is opened on a clock-glass, washed by means of a stream of hot water from a wash-bottle, and allowed to dry in the air.

If annatto is present, an orange tint is imparted to the filter-paper, and its presence is confirmed by adding stannous chloride solution or citric acid solution (5 per cent), when a pink colour is developed.

Detection of blood in milk

A pink colour in milk may be due to the presence of blood. This may be detected by warming a portion of the sample to 50° C and then centrifuging it; if blood is present a bright red deposit is seen at the bottom of the tube. The deposit may be examined microscopically, but it is usually found that the blood corpuscles have become considerably disintegrated. As a confirmatory test, a portion of the red deposit should be treated with a drop of glacial acetic acid on a microscope slide, a cover-glass placed over the mixture, and the acetic acid evaporated very gently over a small flame. When nearly dry, the slide should be examined under the $\frac{1}{6}$-inch objective; the presence of characteristic brown rhomboid crystals of haemin hydrochloride will indicate blood.

(4) DETECTION OF SUCROSE IN MILK

Cotton gives the following test for sucrose in milk. Ten ml of the milk are mixed with 0·5 g of powdered ammonium molybdate and 10 ml of dilute hydrochloric acid (1 : 10). In a second tube 10 ml of milk of known purity, or 10 ml of a 6 per cent solution of lactose, are treated similarly. The two tubes are placed in a water-bath and the temperature raised gradually. At about 80° C the milk, if adulterated with sucrose, assumes an intense blue colour, whilst the genuine milk, or solution of lactose, remains practically unaltered. On boiling, these latter also turn blue, but to a less extent than the adulterated milk.

This test will not detect with certainty 0·2 per cent of sucrose. De Koningh modified the test by adding 2 ml of a saturated solution of ammonium molybdate and 8 ml of hydrochloric acid (1 : 8) to 10 ml of the milk, raising the temperature gradually to 80° C (not above) and keeping it at that level for 5 minutes. Under these conditions, 0·05 per cent of sucrose could be detected.

Leffmann and later Gawalowsky employed the reaction with sesame oil and hydrochloric acid to test for sucrose. One ml each of sesame oil and hydrochloric acid are mixed with a little of the filtrate produced by adding strong hydrochloric acid to milk, and the mixture shaken actively for a few moments; if a red colour is produced on standing for 30 minutes the presence of sucrose may be assumed.

Rothenfusser (1910) described a test for sucrose which consists in precipitating the lactose, proteins and fat by adding 10 ml of freshly-prepared ammoniacal lead acetate solution to 10 ml of milk (430 g of neutral lead acetate and 130 g of litharge are boiled for half-an-hour with 500 ml of water; after cooling, the decanted liquid is diluted to specific gravity 1·15; 2 volumes of this are mixed with 1 volume of 0·959 ammonia). To 4 ml of the filtrate 8 ml of diphenylamine reagent are added (20 ml of a 5 per cent solution of diphenylamine in 95 per cent alcohol + 60 ml glacial acetic acid + 60 ml hydrochloric acid, specific gravity 1·17 and 60 ml of water) and the liquid heated in the water-bath for ten minutes. A blue colour indicates sucrose.

This test will detect 0·05 per cent of sucrose; lactose and dextrose, however, interfere and must, therefore, be removed before the test is carried out.

Cayaux's original resorcinol test is as follows. One ml of hydrochloric acid and 0·1 g of resorcinol are added to 15 ml of milk, and the whole brought to boiling-point in the water-bath; in the presence of sucrose a red colour is produced. Using this test, it was found possible to detect 0·2 per cent of sucrose.

Elsdon (1918) states that a more delicate reaction is obtained if the above test is carried out as a spot reaction as follows. One ml of 3N hydrochloric acid and 0·5 g of resorcinol are added to 15 ml of milk. Five drops of the resulting mixture are placed in one of the depressions of a white spot tile and are then evaporated to dryness on a water-bath. The presence of sucrose is indicated by a red coloration. By this means it is possible to detect 0·02 per cent of sucrose.

For the determination of sucrose see p. 371.

CHAPTER 19

DETECTION AND DETERMINATION OF ENZYMES

TESTS FOR ESTIMATING THE DEGREE OF HEATING OF MILK

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(1) DETECTION AND DETERMINATION OF ENZYMES IN MILK

Detection of peroxidase

On mixing tincture of guaiacum and a little hydrogen peroxide with milk, a characteristic blue colour is produced when peroxidase is present.

Storch's test (1899)

To about 5 ml of milk add a few milligrams of powdered paraphenylene-diamine, and shake well; on the addition of a drop or two of a 10-volume solution of hydrogen peroxide, fresh milk gives a blue coloration; pasteurised milk gives a similar reaction, not, however, so marked; while sterilised milk gives no coloration within ten minutes. A mixture of " sterilised " and fresh milk will give similar results to pasteurised milk.

The hydrochloride of metaphenylene-diamine may be substituted with advantage for the para-compound. The coloration is paler, and not quite so quickly developed. By shaking with an equal volume of amyl alcohol the blue substance is dissolved in the alcohol layer, and the test is thus rendered more reliable in the presence of substances which modify the tint (e.g. formaldehyde).

Other substances, such as quinol or tincture of guaiacum, may be used; of these the one recommended by Saul, and found most effective by Richmond, is " ortol ", which is sold as a photographic developer. This gives a fine red colour with unboiled milk. Benzidine, dissolved in a little acetic acid, as recommended by Wilkinson and Peters, or benzidine hydrochloride, recommended by Leffmann, may also be used with advantage.

If characteristic colours are obtained by these tests, it is proved that the milk has not been heated above 70° to 80° C, as the peroxidase which gives rise to the reaction is destroyed at this temperature.

The exception to the above statement is milk which has been treated with hydrogen peroxide (Budde's process). Peroxidase is destroyed by an excess of hydrogen peroxide, with the result that no colours are produced in the above tests by milk so treated.

In order to differentiate between the cause of negative results, it is necessary to determine the soluble albumin, as milk treated by Budde's process will still contain an abundance of soluble albumin, while milk heated to 80° C will contain practically none.

Determination of catalase

Several methods are available for the determination of this enzyme.

(*a*) Direct determination of the amount of gas liberated from a mixture of milk and hydrogen peroxide.

(*b*) Indirect determination of the amount of hydrogen peroxide remaining after reaction with the catalase, depending upon either titration with potassium permanganate, or titration with thiosulphate of the iodine liberated from potassium iodide by the peroxide. Anderson and MacWalter (1937) have investigated several methods and ultimately decided to adopt the following iodometric procedure as being the most satisfactory.

Twenty ml of milk are pipetted into a 6 × 1 in. boiling-tube, followed by 10 ml of 0·2 N hydrogen peroxide, and the tube is gently twirled to mix the contents. The tube is then plugged lightly with cotton wool and placed in a water-bath at 23°. After 4 hours the contents are washed into a flask, diluted to 150 ml with water, and 2 g of potassium iodide are added, followed by 30 ml of 18N sulphuric acid. After 10 minutes, the liberated iodine is titrated with 0·1 N thiosulphate, using starch as indicator. A control experiment is always made, using milk which has been heated in a boiling water-bath for 30 minutes. The results are expressed as mg of oxygen liberated per 100 ml of milk.

Zaykowsky and Aleksejeff's (1930) method, as modified by Anderson and MacWalter, is as follows. Two ml of milk, diluted to 100 ml, are allowed to react with 5 ml of 3 per cent hydrogen peroxide for 30 minutes. The residual peroxide is titrated with 0·1 N potassium permanganate and compared with a similar determination carried out on a boiled-milk control; the difference represents the hydrogen peroxide decomposed by the catalase. This method was considered unsatisfactory by the latter workers for several reasons, one being the fact that the reaction did not appear to be complete in the time stated.

Koning's (1907) method consists in taking two stoppered flasks of 250 ml capacity and placing 5 ml of milk in each; to one, 6 drops of hydrochloric acid (1 : 1) are added to destroy the enzyme, and 5 ml of 1 per cent hydrogen peroxide are added to each, and the flasks kept at 38° C for two hours; 10 ml of strong hydrochloric acid are added, shaking well, then 10 ml of 10 per cent potassium iodide solution, and after 15 minutes 100 ml of water. The liberated iodine is titrated with 0·1 N thiosulphate with starch as indicator; owing to the adsorption of iodine by the casein, the titration takes about half-an-hour. The difference between the two titrations, calculated as ml 0·1 N per 100 ml of milk, gives the catalase activity, and the normal figure is less than 5.

A less accurate catalase test may be made by treating milk with one-third of its volume of 1 per cent hydrogen peroxide solution, and measuring the volume of gas which is given off. Lobeck's catalase tube (Fig. 4) is very convenient for this, though any other form of measuring apparatus may be used. For the estimation, the measuring cylinder is filled with water through the opening *d*, the cover of which is then screwed down. The opening *b* (for cleaning the tube) is closed with a rubber stopper, and the chamber *A* is charged, through the opening *c*, with 15 ml of milk and 5 ml of 1 per cent hydrogen peroxide solution (or 9 ml of milk and 3 ml of hydrogen peroxide). The tube is now held at *f* and *d* and shaken with a pendulum motion, and the cover to *c* screwed rapidly down. The tube is then placed in water at 25° C up to the level of *c*, and the place where the water stands in the upper tube noted at once; the tube is shaken from time to time during two hours, and the volume read off after two hours; the difference of the reading gives the liberated gas. A volume of 25 ml of gas per 100 ml of milk is about the limit for normal milk.

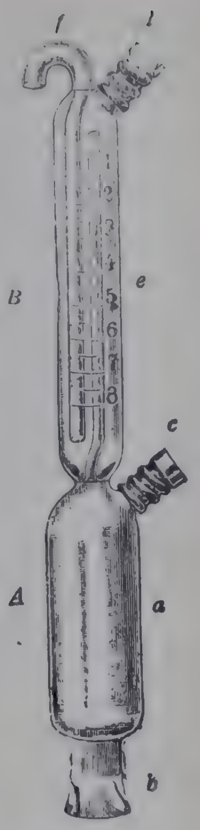

Fig. 4
Lobeck's catalase tube

Determination of phosphatase

The steady increase in the proportion of milk which is pasteurised or otherwise heat-treated has naturally resulted in increasing attention to the methods of testing the efficiency of the heat-treatment. Early methods were based on destruction of peroxidase, rate of rise of cream, proportion of albumin and globulin precipitated, etc. Most of these, however, were applicable for heat-treatments more drastic than pasteurisation as now defined in this country (145° F for 30 min. or 161° F for 15 sec.) and they may all now be regarded as obsolete and superseded by the phosphatase test, which is more precise and quantitative and (the latter enzyme being slightly more difficult to destroy than *Mycobacterium tuberculosis*) therefore eminently suitable for the control of pasteurised milk at the present day.

Phosphatase is always present in raw milk, but is destroyed at the temperature necessary for efficient pasteurisation. If phosphatase is present, it therefore follows that the milk is unheated, has been inadequately heated, or has been contaminated after the heating process by raw milk.

The test now used was originated by Kay and Graham (1935) and further investigated by Anderson *et al.* (1937). The applications of this and other modifications of the test have been very fully described by Kay *et al.* (1939). The principle of the test is that when milk which contains phosphatase is incubated with disodium phenylphosphate, free phenol is liberated, and this, which may be determined colorimetrically, is an approximate measure of the phosphatase content of the milk.

Destruction of phosphatase by heat

Lear and Foster (1949) have confirmed that the heat inactivation of phosphatase is a monomolecular reaction. The *Z*-value from the temperature–log time curve was 4·9° C. They obtained values as follows—

Temperature °*C*	*Holding time* (*min.*)	*Phenol* *p.p.m.*
60	40	0·35
61	35	0·17
62	21	0·33
63	15	0·47
64	7	0·64
65	7	0·54
66	3·5	0·84
67	2·5	0·53
68	1·25	0·39
69	1·00	0·78
70	0·50	0·55
71	0·30	0·30

Sanders and Sager (1948) have described their laboratory pasteuriser with which they have shown that for the destruction of phosphatase in liquid dairy products, temperatures plotted against the logarithms of the time of heating give a straight line. For the destruction of the enzyme in skim milk a temperature of about 0·7° F (0·39° C) less than in whole milk, and for cream about 0·7° F higher was required. Ice-cream mix required about 4·5° F (2·5° C) higher and a heating period three times as long. In cheese the enzyme was destroyed in 13 min. at 130° F (54·5° C) and in 40 sec. at 140° F (60° C). Addition of alkalis, emulsifiers and lactose increased the stability. In milk phosphatase was most stable at pH 6·5–7·4.

Haller *et al.* (1942) have shown that 5 min. at 143° F (61·7° C) is sufficient to inactivate the phosphatase in goats' milk. The test as used for cows' milk is therefore not suitable.

For the official test, which is Neave's modification of the Kay-Graham test, see p. 541. The test was worked out for low-temperature long-time or " holder " pasteurised milk, as officially defined in Great Britain, i.e. milk " retained at a temperature of not less than 145° F and not more than 150° F for at least 30 minutes and . . . immediately cooled to a temperature of not more than 55° F ".[1]

Pasteurisation may also be carried out by the high-temperature short-time (H.T.S.T.) method. The phosphatase test is equally suitable for H.T.S.T. pasteurisation as defined in England and Wales (see p. 310).

It will, of course, be understood that while the phosphatase test as applied to pasteurised milk provides a very sensitive means of controlling efficient pasteurisation, it does not entirely dispense with bacteriological examination, as it is incapable of detecting bacterial contamination, other than that due to raw milk, which might occur after the pasteurising process.

Recent investigations and other techniques

Aschaffenburg and Mullen (1949) have developed a quicker and simpler form of the test using disodium-*p*-nitrophenyl phosphate as a substrate. They report an optimum pH of 9·8 to 10, an optimum concentration of M/100, and an optimum temperature of 37°. The hydrolysis proceeds at constant velocity for the first half-hour and then falls off. The yellow colour of the *p*-nitrophenol in alkaline solution is finally estimated after 30 min (quick test) or 2 hr. (more accurate test). This new test is thus quicker than the ordinary 24 hr. test and avoids the complications and false positives so easily given when using the Folin reagent.

Tintometer discs have been devised for this new test by Tramer and Wight (1950). They have given values for the 30-minute and two-hour procedures of Aschaffenburg and Mullen (1949) corresponding to the 2·3 Lovibond blue units for the original Kay and Graham test. See also Wight and Tramer (1952).

Sodium phenolphthalein phosphate has been proposed as a substrate by Stiven (1947) in a method which eliminates protein precipitation and filtering. The liberated phenolphthalein is detected by its pink colour in alkaline solution. Andersen and Hartmann (1947) have criticised the Scharer phosphatase tests. Milk contains up to 3 mg phenol per litre ≡ 1·5 blue units in the Scharer test. They have further shown that an inhibitory effect can be detected in milk, possibly due to amino acids and ascorbic acid. The blue colour developed is

[1] Milk (Special Designations) Order, 1936, S.R. & O., 356.

not proportional to the phenol present, and Hartmann (1947) has proposed a new modification based on the phosphatase activity of the water-soluble protein fraction. Kelly (1942b) has searched for an activator to shorten the time of the phosphatase test. Only zinc at a high ionic dilution appeared to accelerate the reaction. Sanders and Sager (1949) have investigated a number of preservatives for use with the phosphatase test and classify them in the following order of increasing inhibitory effect on the enzyme: 1·5 to 2 per cent chloroform, 3 to 3·5 per cent toluene, 1·5 to 2 per cent borax, 0·01 to 0·03 per cent formaldehyde, 0·05 to 0·1 per cent mercuric chloride and 0·06 to 0·15 per cent hydrogen peroxide. They recommend 1·5 to 2 per cent chloroform for liquid dairy products, and for solids 1·5 to 2 per cent of the volume of the container.

It has been shown that some types of water can bring about decomposition of the phenyl-phosphate buffer (New Zealand, 1945).

Cream—Brown and Elliker (1942) obtained good agreement between the Kay-Graham-Neave and the Scharer tests for holder pasteurisation of cream. The phosphatase values of flash-pasteurised cream increased somewhat in storage. With " vacreated " neutralised cream the two methods gave rather different results. Wiley *et al.* (1941) have found that " flashing " cream in a Vacreator at about 200° F (93·3° C) does not destroy phosphatase completely. Flash-pasteurised cream appears to contain some bound phosphatase.

Cheese—Thibodeau (1947) has modified the Spicer and Price method for small samples of cheese. Gilcreas (1947) finds that the Sanders and Sager method for phosphatase in cheese is preferable to a modified Kay-Graham method as the more specific Gibbs reagent is not affected by tyrosine formed in the ripening of cheese. Milk heated to 143° F (61·7° C) for 30 min. or to 161° F (71·7° C) for 15 sec. gave cheese which passed the test. An admixture of 0·6 per cent cheese made from raw milk could be detected. Sanders and Sager (1947) have further reported that their modification for cheese can be used for cream, butter, buttermilk, different types of cheese, whey, ice-cream, sherbet and chocolate drink. When testing soft cheese, samples should be taken from the centre as micro-organisms on the surface may produce phosphatase.

Kosikowsky and Dahlberg (1949) have described a phosphatase test for cheese involving incubation in carbonate-bicarbonate buffer at pH 9·55, precipitation by trichloracetic and hydrochloric acid, ether extraction of the phenol and colorimetric estimation either by the Folin-Ciocalteu or the 2.6 dibromoquinone chloramide reagent. They suggest that any value greater than 0·02 mg phenol per 0·5 g of cheese indicates inadequately pasteurised milk or an admixture of more than about 0·1 per cent raw milk.

Kosikowsky and Dahlberg (1948b) have elaborated a technique for cheese in which the phenol is extracted with acid ether and tested free from such interfering substances as tyrosine and tyramine.

Sanders (1948) describes suitable modifications of the phosphatase test for all the usual dairy products, and Andersen (1947) has shown that Vestesen's phosphatase test can be used for whey with bichromate as a preservative.

The methylene blue reduction test

This is the official test for the bacteriological control of Tuberculin Tested milk and Accredited milk prescribed by the Milk (Special Designations) Order

1936 and also in the Milk (Special Designations) (Raw Milk) Regulations 1949. The test is now also used for pasteurised milk after storage.

The technical details of the prescribed tests are given on pp. 537 and 539.

Determination of lipase

E. C. V. Mattick and Kay (1938) have suggested the following method for the determination of tributyrinase; this method has the advantage over those previously described in that it takes only 6 hours to carry out. There is still some doubt whether true milk lipase and tributyrinase are identical, but Mattick and Kay's investigations indicate that they are at least extremely similar.

In order to preserve a pH of 8·5, which is the optimum for the activity of tributyrinase, the solution is buffered with sodium diethyl barbiturate, and in order to avoid the difficulty of enzyme and substrate not being soluble in the same phase, tributyrin is used as substrate; this substance has a solubility in the buffer solution, under the conditions of the determination, of at least 1 in 2,800.

For a determination, 100 ml of 0·1 N sodium diethyl barbiturate, 0·5 ml of pure tributyrin, and 20 ml of the milk or other enzyme-containing material are placed in a 150-ml Erlenmeyer flask. No preservative is necessary as the reaction period is relatively short; bacterial growth was, however, checked by plating before and after the determinations on standard milk agar, but was found to be negligible.

As soon as the reaction mixture is made up, 20 ml are at once removed and transferred to a 250-ml round-bottomed pyrex flask and steam-distilled after adding exactly 10 drops of phosphoric acid (A.R. sp. gr. 1·75); this stops the reaction and increases the acidity to about pH 1·6. This represents the blank determination.

The rest of the mixture is incubated at 37° C with gentle continuous shaking in a water thermostat, and at intervals up to six hours aliquots are removed, acidified, and steam-distilled.

The distillation apparatus must be all-glass to obtain consistent results, and must be steamed out before a determination until 100 ml of distillate, which is collected in a cylinder fitted to the end of the condenser with a two-holed rubber stopper which also carries a soda-lime tube, gives a titration of less than 1 ml of carbon-dioxide-free 0·01 N sodium hydroxide.

In a determination, 100 ml of distillate are also collected, and this is titrated with 0·01 N NaOH to phenolphthalein in the collecting cylinder, using a glass plunger to facilitate mixing. The bulk of liquid in the reaction flask is kept as near 20 ml as possible throughout the distillation by adjusting a small burner under the flask. The amount of enzyme may be returned as the number of ml of 0·01 N butyric acid produced in 20 ml of the reaction mixture, the result being corrected for the amount obtained in the initial " blank " experiment, determined immediately the reaction mixture was made up.

Rice and Markley (1922) have suggested the use of cream as substrate for the determination of the lipolytic activity of milk. This method has the obvious advantage that the natural fat of milk is used and, therefore, any activity will be due to a true lipase.

Cream containing 50 per cent fat is treated with sufficient cane sugar to saturate the water (two parts of sugar to one part of water) and is then boiled in order to dissolve the sugar and destroy any enzymes present. The sugar is added to prevent the growth of fat-splitting micro-organisms after the addition of the sample. After cooling, the enzyme-containing material is added and the mixture incubated at 38° C for periods up to 30 days, depending on the amount of enzyme present (should the enzyme be present in considerable amount, it is soon possible to detect the odour of butyric acid). A control experiment, using the same amount of boiled enzyme solution, is carried out at the same time.

The acidity in millilitres of 0·1 N butyric acid is determined by titrating with 0·1 N NaOH to phenolphthalein after diluting 10 g of the sample to 50 ml, and is corrected for the acidity initially present in the reaction mixture. Disadvantages of this method are that experiments take several days or weeks to complete, and that even in the presence of cane sugar the development of micro-organisms may occur.

(2) TESTS FOR ESTIMATING THE DEGREE OF HEATING OF MILK

These can be divided into tests for (*a*) the inactivation or destruction of enzymes, (*b*) the determination of soluble albumin, and (*c*) changes in the physical condition of the milk.

It has already been pointed out that the various milk enzymes are affected to a varying degree by the application of heat, for example: peroxidase is destroyed by heating to between 70° and 80° C, depending on the duration of the treatment; phosphatase is destroyed under varying conditions between 63° and 75° C; Schardinger's enzyme is destroyed by heating to 75° C for 20 minutes; and catalase is destroyed by heating to 90° to 92° C for 20 to 30 minutes. It is therefore possible, by testing for the various enzymes, to get a rough idea of the temperature to which milk has been heated.

A summary of the time–temperature conditions necessary for the destruction of some milk enzymes is given in Table 19.1.

Table 19.1—Time–temperature conditions required for destruction of enzymes

Amylase	57°	1 hr. (*b*)
	58°	30 min. (*b*)
	59°	14 ,, ,,
	60°	6 ,, ,,
Lipase	62°	50–115 min. (*b*)
Phosphatase	60°	1 hr. (*b*)
	62°–63°	20–30 min. (*b*)
Peroxidase	81°	60 min. (*a*)
	82°	20 min. (*a*)
	70°	2 hr. (*b*)
	72°	20 min. (*b*)
	74°	7 ,, ,,
Schardinger	78·5°	60 min. (*a*)
	80°	10 ,, ,,

(*a*) Pien (1945)
(*b*) Hajek (1950)

Tests for peroxidase were probably those most frequently employed before the year 1935 for the detection of milk heated to above 80° C. It should be remembered, however, that this enzyme is also destroyed by excess of hydrogen peroxide, and in order to differentiate between this and heat treatment it is necessary to determine the amount of soluble albumin present (see below). Positive results are obtained in tests for peroxidase on mixtures of heated and raw milk, and it is not possible therefore by this means to detect the presence of heated milk in raw milk.

The phosphatase test of Kay and Graham, as used for the control of pasteurised milk, is probably based on the enzyme reaction which, at the present time, will give the most definite information as to the heat treatment of milk. This test has been very fully investigated and has been applied with unqualified success during the last few years to pasteurised milk from numerous sources throughout the country. A sample of milk which gives no reaction for phosphatase under the conditions of the test has been heated to 75° C for a few seconds, or at least to 63° C for a longer period. In addition, the test is capable of detecting the presence of 0·25 per cent of raw milk in pasteurised milk.

The phosphatase reaction and tests for other enzymes have been described in the earlier pages of this chapter.

When milk is heated for a limited time to about 70° C, a change takes place in a considerable proportion of the albumin, and under the same conditions at 80° C the whole of the albumin is altered. It is not actually precipitated, but is converted into a form which is precipitated by acids, magnesium sulphate, and other precipitants of casein. Table 19.2 by C. H. Stewart shows the percentage of albumin found in milk raised to various temperatures.

Table 19.2—Percentage of albumin in milk at various temperatures
(*C. H. Stewart*)

Time of heating	Soluble albumin in fresh milk	Soluble albumin in heated milk
10 minutes at 60° C	0·423	0·418
30 ,, ,, ,,	0·435	0·427
10 ,, ,, 65° C	0·395	0·362
30 ,, ,, ,,	0·395	0·333
10 ,, ,, 70° C	0·422	0·269
30 ,, ,, ,,	0·421	0·253
10 ,, ,, 75° C	0·380	0·070
30 ,, ,, ,,	0·380	0·050
10 ,, ,, 80° C	0·375	none
30 ,, ,, ,,	0·375	none

The determination of the true albumin in solution, therefore, offers valuable confirmation of the results obtained from enzymic reactions for heated milk.

Methods for the determination of albumin are given in the section on " Determination of proteins ".

It has long been recognised that the cream of heated milk tends to separate more slowly than that of raw milk, and this phenomenon is accentuated by dilution with water. Orla-Jensen's (1932) " creamometric " method depends on this principle, and is carried out by heating the sample for 5 minutes at 50° C and then placing 10 ml of undiluted milk and 5 ml of milk and 5 ml of water in cream-butyrometer tubes (Hoyberg or Gerber); after standing for 2 hours at 12° to 15° C the layer of cream is read off. Addition of a colouring-matter (e.g. the Storch reagent) gives a well-defined layer. The ratio *A*, viz. 2 × thickness of cream in dilute milk/thickness in undiluted milk, should exceed 1 for raw milk, and is less than 1 for milk pasteurised at a low temperature. Should the undiluted milk give only a thin layer of cream, the fat content of the undiluted milk is taken as the denominator. This gives the ratio *C* which is 3·9 to 10 for raw milk, 0·1 to 3·7 for " holder " pasteurised milk, and 0 to 0·3 for milk heated momentarily to 75° C. The effect of homogenised milk on this test is not stated, but Davies (1939) states that the test is unsatisfactory with milk containing large fat globules, such as milk from Jersey cows.

Stoppel (1937) has modified the Orla-Jensen test to enable it to indicate the presence of small amounts of raw milk in pasteurised milk by increasing the amount of sample taken, altering the dilution, and allowing it to stand for a much greater length of time. The test is carried out as follows: Thirty ml of the sample are heated for 5 minutes at 50° C in a 100 ml flask and then quickly cooled. The flask is completely filled with water and is connected by means of rubber tubing, about 5 cm long, with the lower end of a Hoyberg butyrometer tube also filled with water (it is advisable to include about 1 ml of air to facilitate mixing). The whole apparatus is now inverted several times to ensure complete mixing of the contents (120 ml). After allowing to stand at 12° to 15° C for 20 hours, the cream layer is read off. Factor *C*, above, is calculated by multiplying the depth of the cream layer by 4 (the dilution) and dividing by the fat content of the undiluted milk. The average values of the factor *C* found by Stoppel for additions of 0, 5, 10, 15, 20 and 30 per cent of raw milk to pasteurised milk were 2·8, 6·0, 16·4, 28·8, 41·1 and 55·9 respectively.

Fig. 5 gives a general picture of the effect of temperature on the bacteria, chemical constituents and physical properties of milk. Richmond (1920) states that at near 100° C calcium salts are deposited in small amount, and, by keeping at this temperature for some time, slight oxidation sets in, with the production of traces of formic acid and a marked reduction in the rotatory power of the milk-sugar; a brown colour is produced at the same time.

If the surface of milk be exposed freely to the air, a skin forms at temperatures exceeding 60° C. This has been stated to consist of casein, but has not the properties of this substance; it is partly of a protein character, and there is some reason to suppose that it is an oxidation product. It contains all the constituents of the milk in a concentrated form, and as it is not produced if the vessel in which the milk is heated is covered, its formation is probably at least partially due to evaporation. The taste and smell of milk are changed by heating to above 70° C.

So far we have dealt solely with the differentiation of raw milk from heated milk. The differentiation of " sterilised " milk from " pasteurised " milk is

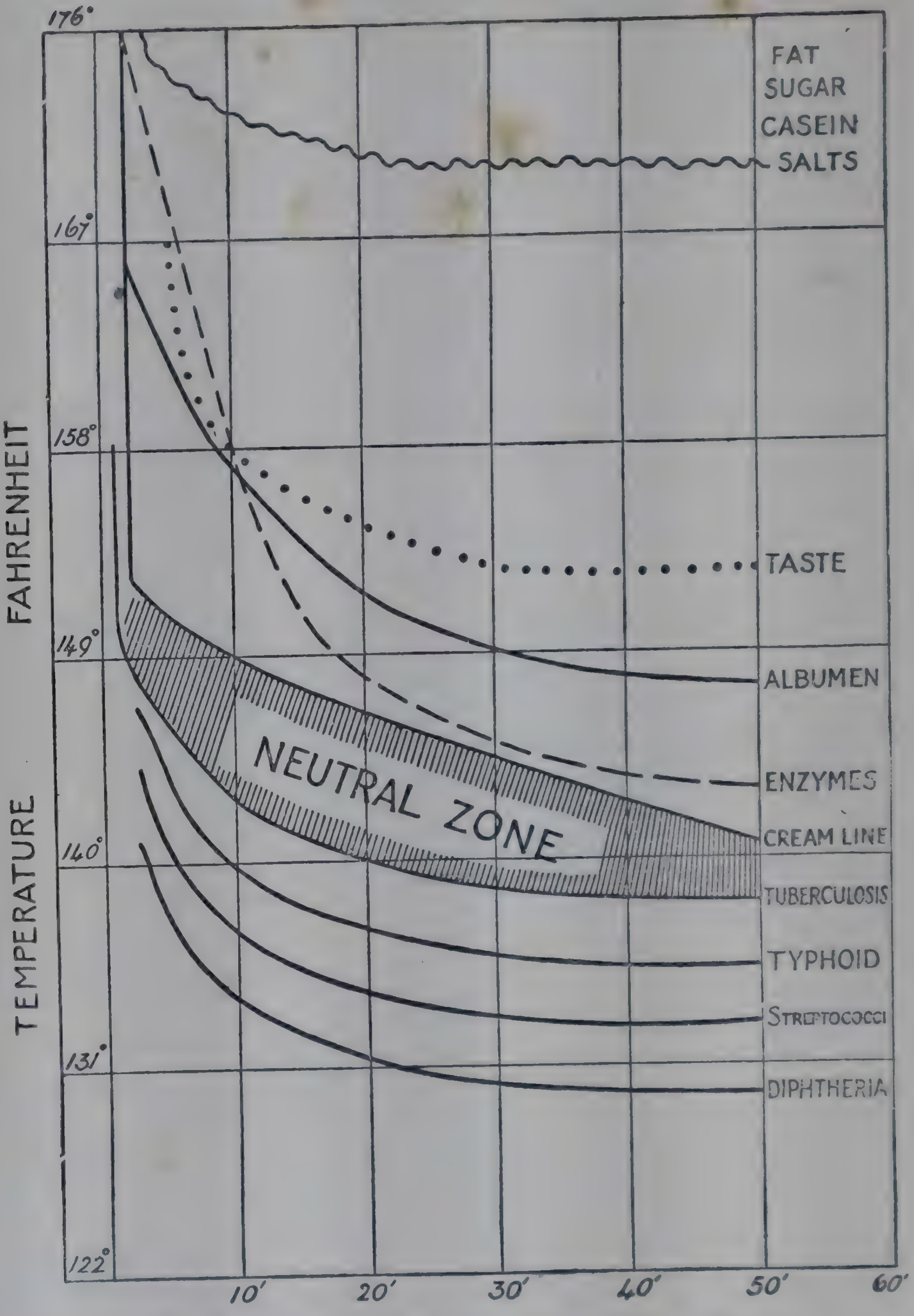

Fig. 5—To illustrate the action of heat on milk

also of considerable importance. Sterilised milk is heated to a higher temperature than pasteurised milk, and is usually easily recognised by the characteristic odour and taste of boiled milk and by the equally characteristic glossy appearance of the dried total solids.

The most marked characteristic distinguishing sterilised milk from new milk is the condition in which the albumin exists. As previously stated, it is probable that albumin exists in milk in combination with a base; on heating milk, no visible coagulation of albumin takes place, but on acidifying, or saturating with magnesium sulphate, the albumin separates with the casein. The albumin appears to be changed from a soluble to a colloidal form. (Not more than 0·1 per cent of albumin is found in the soluble form in sterilised milk.) The casein separates on acidifying in a more finely-divided state. This property of albumin has been applied by Aschaffenburg to form the basis of the *turbidity* test for sterilised milk (see p. 544).

If the milk has been heated to 100° C or a higher temperature for any length of time, the rotatory power of the milk-sugar undergoes a serious reduction, the cupric-reducing power not changing to any appreciable extent. The milk also assumes a slight brownish colour, due probably to the formation of a "caramelised" body of low rotatory power. Furthermore, the cream rises with extreme slowness; in three hours, practically no cream is observed on the surface of the milk; and after six hours, the layer is only about one-tenth of that given by new milk. If sterilised milk be allowed to stand for twenty-four hours or more, the bulk of the cream will rise to the surface, but the quantity will be less than that yielded by new milk; the cream will, however, contain a distinctly larger percentage of fat, about 40 per cent as against less than 30 per cent in the cream yielded by new milk.

The diminished yield of cream is a property shared also by milk which has been pasteurised by heating to about 70°, but the rate of rise of cream in pasteurised milk is fairly rapid; practically the same amounts are found in three hours as in six hours. The total quantity of cream from pasteurised milk is about half that of fresh milk.

The figures in Tables 19.3 and 19.4, which were obtained by Boseley and Richmond, will illustrate the above facts. Table 19.4, showing the behaviour of new milk, is given for the sake of comparison. The samples 1 to 5 are from the same cows respectively which yielded the samples with corresponding numbers in the tables illustrating the behaviour of sterilised milk.

Condensed unsweetened milk, which has been diluted to the original volume with water, has all the analytical characteristics of sterilised milk.

Table 19.3—Percentage composition of sterilised milk

Sterilised milk allowed to stand for six hours

No.	Fat in milk	Cream	Fat in cream	Fat in skim-milk
1	4·30	1·3	23·3	4·05
2	3·80	0·8	22·3	3·67
3	4·25	1·7	20·7	3·95
4	4·10	1·9	24·7	3·70
5	5·35	2·8	31·4	4·60
6	3·62	0·3	—	—

Table 19.3—Percentage composition of sterilised milk *(contd.)*

Sterilised milk allowed to stand for twenty-four hours

No.	Fat in milk	Cream	Fat in cream	Fat in skim-milk
1	4·30	7·0	46·8	1·10
2	3·80	6·0	41·8	1·37
3	4·25	8·8	39·0	0·90
4	4·10	8·7	41·0	0·58
5	5·35	11·1	41·4	0·85
6	3·62	0·8	—	3·48

Table 19.4—Percentage composition of new milk

New milk allowed to stand for six hours

No.	Fat in milk	Cream	Fat in cream	Fat in skim-milk
1	4·05	9·2	17·4	2·70
2	4·20	11·2	16·5	2·65
3	3·90	9·8	15·9	2·60
4	3·70	9·8	18·0	2·15
5	4·45	13·5	16·8	2·30

The cream separates rather less readily even than in sterilised milk. This is partly due to the fact that it has been condensed, but chiefly on account of the sterilising process that it has undergone. No. 6 in Table 19.3 was diluted condensed milk. There appears to be no good method of discriminating between condensed milk diluted with water and sterilised milk. If water containing large amounts of nitrates has been used for diluting the condensed milk, a strong diphenylamine reaction will indicate the probability that water has been added.

CHAPTER 20

DETERMINATION OF VISIBLE DIRT IN MILK

Visible dirt in milk may consist of particles of dung, siliceous matter, iron oxide, straw, hay and other vegetable debris, from road dust; fibres of cotton, wool, etc. from domestic dust; cow hairs; and cells such as epithelial cells, red blood-cells, pus cells, and leucocytes. The presence of dung, whilst it may be due to the ingress of road dust, usually indicates dirty conditions of milking, and its determination is therefore of considerable importance in the hygienic control of the milk supply.

It should be remembered that, with dung, the visible dirt represents only a comparatively small proportion, probably less than 20 per cent by weight, of the total contamination, the greater and more injurious proportion consisting of soluble matter and bacteria. For this reason the practice of straining dirt from milk by means of a sieve containing a filter pad, as it is poured into the churn after milking, is a procedure which merely removes the visible signs of contamination and leaves the milk, from a hygienic point of view, still in a " dirty " condition. As long as the system of allowing the use of sieves is permitted, there is very little incentive for the milker to adopt stringent " clean-milking " principles, which require considerable time and trouble to put into operation, when the same results can be apparently simulated by merely passing the milk through a sieve. It will be obvious from the above that no milk can be considered " clean " unless it passes bacteriological and other tests, in addition to the visible cleanliness test.

The determination of dirt has been the subject of a Report of the Sub-Committee of the Analytical Methods Committee of the Society of Public Analysts (1937) and the methods finally adopted will be described later in this section. The Sub-Committee also made the following recommendation as to the allowable limit of dirt in milk—

> " Although it is not within the terms of reference of the Sub-Committee, the members feel that they are in a peculiarly strong position to recommend such a limit. Any proportion of dung in milk, however small its amount, is objectionable, but there are insuperable difficulties in the way of reporting against milk containing dirt unless the quantity can be determined and expressed in figures. The Sub-Committee considers that clean milk, when examined by their recommended method, contains less than 1 part by volume of moist dirt in 100,000 parts by volume of the milk, and that a limit of 2 parts of moist dirt is the maximum which can be conceded, but in stating this opinion they must not be understood to mean that milk containing less than 2 parts of dirt is necessarily a clean milk. In dealing with milk for the purposes of the Food and Drugs (Adulteration) Act, 1928, the Committee suggests, having regard to the degree of variation in the results obtained in their joint experiments, that it would not be desirable to recommend legal action in the case of an isolated sample of dirty milk unless the amount of moist sediment, determined by the recommended method, exceeds 3 parts by volume in 100,000 parts of the sample."

The simplest method of examining a large number of samples of milk for visible dirt is to allow the samples to remain at rest in their original bottles, placed in a sloping position, for one to two hours. With samples which contain appreciable quantities of dirt, this will then be concentrated mainly in one small area at the bottom of the bottle; these particular samples can then be taken for the actual determination and the remainder marked " free from visible dirt ". Where the samples originally examined contain only a few ounces of milk, it is advisable to take a special sample in which each portion contains at least 1 pint of milk, for the determination. For the purpose of routine testing, mention must be made of the sediment testers, such as the " Gerber ", " Minit" and " Dana " testers. In these a measured quantity of milk of the order of ½ to 1 pint is filtered, in some cases by suction or pressure, through specially prepared filter pads, which retain any dirt on their surface. The pads are then kept for comparison or microscopical examination.

One of the first methods depending on sedimentation and measurement of the volume of dirt is that due to Lowe (1912). About 500 ml of the milk are placed in a tall, cylindrically-shaped vessel, to the bottom of which is attached a glass tube, the lower end of the latter being drawn out to a fine bore so that 0·5 ml occupies about 5 or 6 cm of the constricted portion, which is graduated in 100ths of a millilitre. The milk is allowed to stand overnight, a small amount of formaldehyde being added to prevent souring, and the volume of the precipitate is then noted. This volume agrees closely with the weight of dung originally present.

Tankard (1922) recommended washing the deposit with dilute sodium carbonate solution in order to dissolve precipitated curd, and thereby obtain more complete separation of the dirt from any deposit natural to the milk. The deposit is centrifuged before the reading is taken. In a further communication, Tankard (1923) recommended the use of strong sodium chloride solution of specific gravity 1·18 to 1·20 at 15·5° C, instead of dilute sodium carbonate solution, to separate the white cellular deposit obtained in some samples of milk from the true extraneous dirt. This is effected by mixing the deposit, remaining after centrifuging and draining off the milk, intimately with about 10 ml of the brine. The tube is then allowed to stand upright for 1 to 2 hours, when it will be found that the cellular matter has collected near the surface of the liquid. The tube is centrifuged for 1 minute at a low rate to collect the extraneous dirt completely in the constricted part of the tube, and then the brine with the suspended cellular matter is discarded. The deposit is then washed with distilled water, whirled for a few minutes at 2,000 r.p.m., measured, examined microscopically, and examined for bile acids.

Cow dung, if present, will be seen under the microscope to consist mainly of the undigested cellular fibre of feeding-stuffs which has been " cleared ", i.e. the cell contents have been removed by the action of the digestive juices. The cellular matter is frequently stained yellow with bile.

Bile is most readily detected by a modification of Pettenkofer's test. A portion of the deposit is moistened with sugar solution, dried, and treated with a drop of sulphuric acid. A red colour develops round any particles stained with bile.

The methods finally adopted in the Report of the S.P.A. (1937) Sub-Committee for the determination of dirt in both fresh and decomposed milk consist of sedimentation, followed by subsequent centrifuging of the deposit.

Although the suspended matter in milk tends to fall when the milk is allowed to stand, a certain proportion remains entangled with the fat globules and is kept in suspension, but it was found that by intermittently stirring up the bulk of the milk, this entangled dirt is gradually liberated and falls to the bottom. The treatment required to obtain complete separation of the dirt covers a period of at least 72 hours, and it is therefore necessary to add a preservative to the milk to prevent it curdling.

In order to obtain concordant results when reading the deposit, it was also found necessary to have an accurately calibrated centrifuge tube, and to specify the type and size of the centrifuge and the rate and time of centrifuging.

The details of the S.P.A. Methods are as follows—

Recommended method for the determination of dirt in fresh milk

(1) APPARATUS FOR SEDIMENTATION METHOD

The sedimentation vessel and the centrifuge tubes. The apparatus consists essentially of a standard glass sedimentation vessel of about 650 ml capacity, gradually tapering at its lower end, to which is attached by suitable means one of the standard centrifuge tubes.[1] A long, stout glass rod, carrying a rubber stopper at one end, is required for stirring purposes, and for closing the main vessel at its lower end while the centrifuge tube is disconnected.

The centrifuge tube may be attached to the sedimentation vessel by means of clean, stout rubber tubing (free from detachable powder), the end of the sedimentation vessel fitting loosely inside the mouth of the centrifuge tube; or the centrifuge tube may be ground inside at its open end, to fit the externally-ground lower end of the sedimentation vessel. The British Standard specification, already referred to, requires that the ground-glass joints shall be interchangeable. There should not be, in any part of the apparatus, " ledges " on which particles of dirt may be held up during sedimentation. When a number of determinations have to be made simultaneously, it is convenient to employ a suitable wooden stand holding several vessels and centrifuge tubes. When the sedimentation vessels and centrifuge tubes used are connected by rubber tubing, the tubes should not be supported from below, since any upward pressure will cause the lower end of the sedimentation vessel to be tightly engaged, and fracture may result. If sedimentation vessels and centrifuge tubes with ground-glass joints are used, the tubes should be supported from below by a spiral spring or suitable collar attached to the lowest shelf of the apparatus stand. By this means fracture of the centrifuge tubes is avoided.

The standard centrifuge tubes, which fit into the holders of the ordinary hand-driven centrifuge, are of three sizes—

Tube No.:	1	2	3
Capacity of graduated portion	0·020 ml	0·050 ml	0·200 ml
Subdivisions	0·001 ,,	0·002 ,,	0·005 ,,
Lowest graduation	0·002 ,,	0·004 ,,	0·020 ,,
Approximate bore at graduated part	0·9 mm	1·3 mm	2·4 mm

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[1] The standard sedimentation vessels and centrifuge tubes are specified in B.S. 736—1937, obtainable from the British Standards Institution, Publications Department, 28 Victoria Street, S.W.1. They are of two types, with and without standard ground-glass joints.

Tube No. 2 is to be used for the measurement of volume of all sediments when the volume comes within the limits of the graduations of that tube; Tube No. 3 may be used for the measurement of larger volumes or, for ease of manipulation, during the process of treatment of large volumes of sediment; and Tube No. 1 may be used when very small amounts of finely-divided deposit are to be measured, or occasionally in the second period of sedimentation described in the method, but the after-treatment of the dirt in such a narrow tube is difficult.

The centrifuge tubes must be carefully packed into the holders of the centrifuge. The narrow ends of the tubes tend to rupture the metal buckets, with consequent fracture of the tubes, and this is not prevented by the use of so resilient a packing material as cotton-wool. Fibre thimbles or collars should be used to support the tubes at the shoulders, and distribute the strain on the tubes and metal buckets of the centrifuge.

The standard centrifuge. The standard centrifuge used is one capable of a speed of at least 2,000 revolutions per minute, and of such a size that the measurement across the machine when in action, from outer tip to outer tip of the whirling tubes, is 10½ inches (26·7 cm). A larger centrifuge may be employed if a padded wooden block be inserted into each carrier so that the working distance of 10½ inches is still observed.

Speed of centrifuge. Alternatively, if the centrifuge used has not correct measurements, the proper speed shall be determined by calculation, so that the same centrifugal force is obtained at the tips of the tubes as with the standard form of machine. The speed required in any given case may be calculated from the following formula—

$$\text{r.p.m.} = 2{,}000 \times \sqrt{\frac{10{\cdot}5}{d}} = \frac{6{,}500}{\sqrt{d}},$$

where d represents the diameter of swing in inches.

Any form of centrifuge may be used, so long as the size and relative speed are maintained; when using the standard machine of the prescribed dimensions, the speed must be approximately, but not less than, 2,000 r.p.m. during a five-minute whirling of the tubes containing the dirt from milk. At speeds appreciably lower than 2,000 r.p.m. a higher reading will be obtained, whilst with higher speeds a slightly lower reading may be recorded. From the table of results (see Appendix VI of Report) it will be seen that the ratio of the volumes of dirt at speeds of 2,000 and 2,500 r.p.m. is 100 : 91 respectively. It is therefore necessary to adhere closely to the recommended speed, in order to obtain comparable results between different workers. The speed of a hand-driven centrifuge can readily be ascertained; but with electrical and other types of centrifuge, supplied with bubble indicators, it is necessary to calibrate these indicators, or to mark them at the correct level for a speed of 2,000 r.p.m. or the equivalent speed, calculated as above.

Calibration of the standard centrifuge tubes. The following note may be useful to those analysts who desire to calibrate their own standard centrifuge tubes—

The method of calibration is fully described in the paper by Stott (1937) on "Verification of Centrifuge Tubes used for the Determination of Visible Dirt in Milk". The volume of the dirt with its upper surface in the plane of any graduation mark in a tube differs from the volume of a liquid with its meniscus adjusted to that mark by the volume between the meniscus and the plane. If the position marked 0·010 ml on the tube is to be calibrated, and the weight of water in the tube, filled with the meniscus level at the centre of this mark, is found to correspond with a volume of (say) 0·0115 ml, then there should be deducted from this latter figure the appropriate meniscus correction for the tube in question (e.g. for No. 2 B.S. tube—0·0003 ml), giving 0·0112 ml as the corrected reading for volume to the plane of the 0·010 ml mark. The correction for this tube at the specified mark is therefore + 0·0012, to be added to any reading of dirt measured at that mark. The meniscus corrections must be added when the volumes are obtained from measurements made with mercury. The necessary meniscus corrections for water in tubes of various internal diameters can be interpolated from those given in a paper by More (1929).[1]

Certificates of the National Physical Laboratory for the B.S. tubes state the volumes up to the plane of the centre of the graduation mark. The Sub-Committee recommends the use of centrifuge tubes tested and approved by the National Physical Laboratory.

(2) METHOD. If desired, a portion of the well-mixed sample is first removed from the bottle for the ordinary chemical analysis. A mark is then made with a grease pencil on the side of the main bottle to indicate the level of the milk left in it, and the volume thus noted (representing that amount of the milk used for the determination of dirt) is subsequently measured when the bottle has been emptied. If a one-pint sample of milk is available, the analyst is recommended to make duplicate determinations of dirt by dividing the sample into two approximately equal portions, taking precautions to ensure that the milk is thoroughly mixed and the dirt uniformly distributed during division. Using the No. 2 centrifuge tube, the lowest graduation (0·004 ml) will represent 2 parts by volume of dirt in 100,000 parts by volume of the milk when 200 ml of the sample have been used.

Isolation of the dirt. To the sample, or to each portion of the sample divided as described, a small quantity of diluted neutralised formaldehyde solution or of filtered potassium dichromate solution is added and thoroughly mixed with the milk. Convenient amounts of these preservatives are 0·2 ml of 40 per cent formaldehyde solution in 10 ml of water, or 0·5 g of potassium dichromate dissolved in 10 ml of water, for each 100 ml of milk. Some of the preserved milk is now poured into a standard centrifuge tube and the milk shaken down so that it completely fills the constricted portion of the tube. This tube is next connected to the sedimentation vessel, the apparatus is fixed vertically in a suitable stand, and the remainder of the milk, kept well mixed, is poured into the vessel. Aeration and the consequent formation of froth should be avoided as far as possible. Finally, the receptacle that contained the sample or portion is washed once or twice with small amounts of water, and the washings are added to the milk in the sedimentation vessel. This is covered with a close-fitting inverted glass dish to prevent access of dust, and the milk is allowed to stand for a minimum period of 72 hours. It is advisable

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[1] The water meniscus corrections for the B.S. centrifuge tubes are as follows—

Tube No.:	1	2	3		
Diameter of bore (graduated portion), mm	0·92	1·32	2·28	2·39	2·50
Correction, ml	0·0001	0·0003	0·0016	0·0018	0·0020

to add a small quantity of ether or petroleum spirit, and to keep a thin layer of the solvent on the surface of the milk during the periods of sedimentation, in order to prevent the holding up of dirt in any separated fatty matter. During the time of standing it is imperative that the milk in the sedimentation vessel be stirred intermittently three times daily, at intervals of four hours, with the rubber-ended glass rod. After each stirring, this rod, and the walls of the vessel above the liquid, are washed down with a small quantity of water into the milk, and the rod is removed during sedimentation. The formation of air-bubbles is to be avoided during stirring, which must be sufficiently thorough to disperse the separated cream throughout the milk and to dislodge any dirt deposited on the tapered sides of the sedimentation vessel.

After the completion of the sedimentation period, the rubber-ended glass rod is lowered into the milk until the stopper engages the narrowest part of the sedimentation vessel. The centrifuge tube attached to the lower end of the vessel is removed, leaving the main quantity of milk in the sedimentation vessel. Some of the milk may then overflow, owing to its liberation from the end of the large vessel. In order that no dirt may be lost, any overflowing milk should be caught in a small beaker, and transferred to the centrifuge tube to be used for the second period of sedimentation. As the first centrifuge tube containing the main quantity of dirt will be undesirably full of milk, a portion of this should also be poured off into the second tube, to allow of the centrifuging of the deposit without loss. This second centrifuge tube, with its constricted part completely filled with milk as before, is attached to the sedimentation vessel, the glass rod is withdrawn, and the sedimentation and periodic stirring are continued for a further period of 48 hours or longer.

The centrifuge tube, containing milk and the main part of the sediment, is now transferred to the centrifuge, and the machine spun for two or three minutes at a speed of 2,000 r.p.m. A second tube containing milk or water should be placed in the opposite carrier to balance the machine. The centrifuge tube should be closed with a rubber stopper or cap to prevent access of dust.

Treatment of separated dirt. On removing the tube from the centrifuge, the dirt being wholly collected in the graduated portion, the milk in the upper part of the tube is poured off and added to the main bulk in the original vessel. The milk in the constricted part of the tube is also carefully drawn off as far as possible, without disturbing the sediment, by means of a fine platinum wire which, if allowed to impinge on the upper surface of the milk and the side of the tube when the tube is held in a horizontal position, will cause milk to flow out of the tube. Care should be taken not to produce scratches on the inside of the graduated tube, which may cause breakages when in the centrifuge. The upper part of the tube is carefully washed with a small quantity of distilled water to remove any milk, the tube is nearly filled with water, and the sediment is dislodged from the graduated part of the tube and mixed with the water by stirring with the platinum wire, which is then withdrawn and washed. The tube with its contents is submitted to a few minutes' spinning in the centrifuge, by which means all the sediment will be deposited in the lower end of the tube. The liquid in the wider part of the tube is poured away, and the washing is repeated by adding more water, stirring the dirt up into the water and centrifuging as before. It is essential that all soluble milk solids should be washed away before the next stage of the treatment is begun, and at least three washings with water are necessary.

After as much of the wash-water as possible has been removed by the method already described, a portion of the sediment is examined under the microscope. This portion is carefully returned to the tube, and the whole of the sediment is treated for half-an-hour with 2 ml of cold normal ammonia solution, the sediment being thoroughly mixed with the whole of the reagent by stirring with the wire at once and at intervals of 10 minutes. The tube and its contents are next whirled in the centrifuge for 2 or 3 minutes at a speed of 2,000 r.p.m., when the ammonia solution is run off, with the assistance of a wire, but without disturbing the layer of dirt. The contents of the tube are thoroughly washed two or three times with water by stirring, whirling in the centrifuge after each washing, and running off the wash-waters. After removal of the ammonia solution, the dirt is treated in an exactly similar manner with cold normal hydrochloric acid solution for half-an-hour (see next paragraph), with stirring at ten-minute intervals as before. After centrifuging the tube and its contents to collect the dirt in the narrow portion of the tube, the acid is run off and the dirt is again washed with water. These treatments with ammonia solution, water, hydrochloric acid solution, and water again, are repeated, and the dirt is finally washed with water till practically free from chlorides.

In some exceptional cases, a third treatment with alkali and acid may be desirable, but the time of action of the reagents in such instances should be as short as possible. Circumstances may also conceivably arise which make it necessary to modify the procedure of treatment of the sediment by reagents, and the analyst will of course use his judgment. For example, a milk may contain chalk or other material soluble in mineral acid. The treatment of the sediment with hydrochloric acid would here naturally be inadmissible unless the amount of any acid-soluble constituents were ascertained. The use of alkali causes a slight swelling of the sediment, and the subsequent treatment with acid is necessary as a final stage in the operation. Further, it may be found that some curdy matter is deposited, especially in the second period of sedimentation, and this may not be readily removable by the usual treatment. In such a case, cold normal caustic soda solution, followed by cold normal hydrochloric acid solution and washing with water, may effect the desired separation of the curdy matter from any dirt also present in the deposit.

Measurement of the dirt. In order to get a flat (horizontal) surface for measurement, the tube containing the washed dirt and supernatant water is centrifuged in the standard machine for a short time (30 sec.) at about half-speed, the tube turned half-round in its carrier, and the machine spun gently again. If now the surface of the deposit is higher at one side than the other whilst at this loosely-packed stage, it may be levelled by repeating the spinning after the surface layer has been stirred by a rapid twirling, above the sediment, of the platinum wire slightly bent at the end. The tube containing the dirt is then spun in the centrifuge for 5 minutes at approximately, but not less than, 2,000 r.p.m. The dirt should now be found as a compact mass at the bottom of the tube, when its volume may be measured. It is advisable to make several readings, stirring up the dirt with the water in the tube before each re-centrifuging, and taking the average of the readings. These final measurements must be made immediately on the cessation of the spinning in the centrifuge, or high results may be obtained. The observed reading is adjusted for calibration corrections, and the result is expressed (to the nearest lower half-part) as parts by volume of moist dirt in 100,000 parts by volume of the milk sample under examination.

The additional deposit, if any, obtained during the second period of sedimentation must be treated with the reagents in the same way as the main deposit. As this additional dirt is usually very small in amount, it should either be measured in the No. 1 or No. 2 centrifuge tube or, if too small to reach the lowest graduations in the tubes, it must be added to, and measured with, the main quantity.

If duplicate determinations have been made, the average result should be recorded.

Determination of dirt in sour or decomposed milk

The following is a description of a method which was successfully used by some of the members of the Sub-Committee for the determination of dirt in sour or decomposed milk; further experience of the process may, however, be desirable before it is actually recommended. Occasionally, milk may contain certain organisms, such as *Oidium lactis*, which, in their growth during keeping, produce structures which deposit with the dirt and are resistant to the chemical treatment recommended for the dirt from fresh milk. In many samples, however, this does not occur, and it is possible to obtain a satisfactory measure of the inorganic and vegetable debris from the dirt in the milk.

Gas-formation in sour milk: warning

The attention of analysts is drawn to the fact that samples of milk which have been kept for some time, especially those which have been contaminated with dirt containing gas-forming bacteria, occasionally develop great internal pressure. Such samples, when kept in bottles the corks of which are held in position with thick coverings of sealing-wax or by means of string, must be treated with great care as the bottles may burst, or if the cork is blown out, a large part of the clotted creamy portion of the uneven sample may be ejected. The sample bottle should be surrounded with a cloth until the internal pressure is relieved (see below).

Suggested method of determination of dirt in sour milk

The total volume of the sample must first be ascertained. This can be done by weighing the bottle with the sample and, after the milk has been removed as described below, drying the cleaned bottle and weighing it with the cleaned dried original cork and any paper and sealing-wax which may have been removed during the process. The net weight of milk in grams, divided by 1·03, will give the volume in millilitres of milk in the sample. Marking the upper level with a gummed label or grease pencil is sometimes possible, but, in view of the danger mentioned above, a file should never be used for this purpose.

The sample should be examined for foreign particles. If the milk has been shaken up since souring took place, little evidence of deposit may be found, though dark-coloured particles may be seen throughout the sample. Whilst a finger is firmly pressed on the cork to prevent it from being blown out, all sealing-wax (if any) and dust from the cork and outside of the bottle are removed. The internal pressure is released by thrusting a smooth pointed steel wire, such as a hat-pin, between the side of the cork and the neck of the bottle. If there is a tendency for the milk to froth and rise to the cork, the hat-pin is gently moved about, and any exuded milk is caught in a clean beaker. The cork should not be removed until liquid ceases to ooze out. The milk adhering to the cork is washed off into the beaker, and a clean fresh cork, free from holes which may contain dusty matter, placed in the bottle. Three-fourths of the milk is now transferred to a cylindrical separator, and concentrated ammonia solution (sp. gr. 0·880) measured out to an amount about $\frac{1}{10}$ the bulk of the milk. A part of this ammonia solution is added to the milk in the bottle and the whole shaken vigorously, taking care that no loss occurs by the cork being blown out, or by liquid or dirt escaping from the bottle. The contents of the bottle are now added to the milk in the separator, and the bottle is washed out with the remainder of the ammonia solution.

The separator must now be shaken vigorously, care being taken to prevent the stopper from being blown out. It is important to break down clots and to form a fine emulsion at this stage, in order to assist separation of the dirt and to obviate unnecessary cleaning of the separated particles from clotted milk at a later stage.

The bottle is then successively washed out with clean re-distilled alcohol, ether and petroleum spirit (boiling range, 40° to 60° C), measured out in each case in bulk approximately equal to that of the original milk. After each addition of washing liquid to the milk in the separator, the mixture is thoroughly shaken, as in the Gottlieb process, and is finally allowed to stand one hour to permit of separation of the ethereal (fat) layer. By this time, large particles of sediment may show their presence by settling out. The ethereal layer, which is quite free from sediment, is blown off by means of wash-bottle tubes, and set aside. The lower portion of the milk is then drawn off into several stout round-bottomed tubes (about 6 × 1 in.), which are closed with clean rubber stoppers, and whirled at a minimum speed of 2,000 revolutions per minute for five minutes in a large centrifuge with rigidly-fixed buckets, in which the tubes lie in a nearly horizontal position. The tubes are carefully removed from the machine and, without disturbing the sediment, about two-thirds of the top layer of the milk is poured off into a reserve separator, the tubes filled up again, and the process is repeated until all the alcohol-ammonia-milk layer is treated.

Any emulsion between the milk and the ether layer is retained in the (original) separator, and broken down by shaking with alcohol. The contents of the separator are transferred to one of the 6 × 1 in. tubes, to which is added all insoluble matter in the separator, removed by rubbing with a rod and washing with alcohol. The contents of this tube are treated with alcohol and ammonia solution, and spun in a large centrifuge until the dirt is separated.[1]

Treatment of separated dirt

The main amount of sediment in the large tubes is now concentrated into one tube, in which it is washed twice with water, and all the dirt is then transferred to one of the standard centrifuge tubes. Treatments with normal ammonia and normal hydrochloric acid solutions exactly similar to those adopted with fresh milk are now carried out, and repeated once or twice as found necessary. In some exceptional cases, yeasts, moulds or insoluble protein matter may be present, and it may be found almost impossible to free the dirt from these accompanying substances. Treatment with N caustic soda solution in a boiling water-bath, followed by an acid treatment to counteract the " swelling " effect of the alkali, may assist the clarification in these difficult cases. Care must be taken to heat the tube and its contents gradually in the water-bath, or fracture of the tube may result. After the sediment has been washed with water until free from chlorides, a final centrifugal treatment is given in the standard centrifuge for 5 minutes, at a speed approximating to but not less than 2,000 r.p.m. The volume of moist dirt is expressed as before in parts per 100,000 parts of the milk.

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[1] Instead of carrying out the separation of the dirt by centrifuging the liquid in portions in large plain centrifuge tubes, an alternative procedure, which is to be recommended when a centrifuge with rigidly-fixed buckets is not available, is to transfer the lower layer of milk (the alcohol-ammonia-milk layer) into one or more sedimentation vessels such as are used in the fresh milk method, after having fitted a standard centrifuge tube to each of these vessels. The liquid is then allowed to stand, with intermittent stirring, for 3 days or longer, as already described, and the separated dirt is clarified as detailed in the text.

Sampling

When a sample is being taken especially for the determination of dirt, it is advisable to take a 3-pint sample which should be poured in a jug or measure after carefully stirring the contents of the churn. The contents of the jug should then be kept agitated by means of a large spoon and should meanwhile be poured in small portions at a time into the three sample bottles, until approximately 1 pint is placed in each. This procedure is recommended rather than filling one sample bottle and then going on to the next. It is not advisable to use uncovered corks, nor is it advisable to use sealing-wax to seal over the cork and the neck of the bottle. The procedure suggested in the report is to wrap the cork in waxed paper, and after placing it in the bottle, notch it and tie it on with tape. The whole of the neck of the bottle and cork is then covered with a strong paper cap, which is tied with tape below the flange of the bottle neck. This wrapping is then sealed in two places over the tape, the edge of the paper, and the glass bottle neck.

CHAPTER 21

THE ANALYSIS OF SOUR MILK

Several difficulties arise in the determination of the original composition of a milk which has gone sour. These are due chiefly to the difficulty of obtaining a representative sample, and also to the changes and losses which occur in the solids-not-fat as decomposition proceeds.

If the sample has only recently turned sour, it is often possible, for the purposes of routine analysis, to mix the sample thoroughly by whisking and then, by adding a few drops of strong ammonia and thoroughly mixing, to obtain an approximate figure for the specific gravity. An allowance for the reduction in specific gravity due to the ammonia can be made by determining the gravity on a fresh milk before and after the addition of the same amount of ammonia, and making the necessary adjustment to the specific gravity of the sour sample.

The fat can be determined on a portion of the sample treated with ammonia in the usual way by either the Gerber or the Leffmann-Beam process. Day (1920) found that milks several weeks old gave results by the Gerber process approximately 0·2 per cent higher than those given by the samples when in a fresh condition. The fats separated from the Gerber tests made on the most acid samples had a distinct odour of amyl esters. From experiments carried out on milk to which various lower fatty acids had been added he was of the opinion that the Gerber method, when used for very sour milk, is apt to give misleading results, owing to the formation of amyl esters of the lower aliphatic acids. Lactic acid had no effect and acetic acid little, but butyric acid a large effect.

Total solids may be determined by weighing approximately 10 g of the whisked sample (free from added ammonia) into a tared milk dish, adding a drop of phenolphthalein solution, neutralising with 0·1 N strontia, and proceeding as usual. For each 1 ml of 0·1 N strontia used in the neutralisation, 0·00428 g is deducted from the weight of residue obtained, and the difference is calculated as percentage of total solids. The figure of 0·00428 for each ml of 0·1 N strontia is arrived at through the strontia combining with any free acid, with the elimination of water, in accordance with the following equation—

$$2R.COOH + Sr(OH)_2 = (R.COO)_2\,Sr + 2H_2O.$$

It is thus apparent that, in the solid residue, 1 gram-equivalent of free acid is represented by 1 gram-equivalent of free acid + 1 gram-equivalent of strontium — 1 gram-equivalent of hydrogen; in other words, each gram-equivalent of free acid is increased by 42·8, which is equivalent to 0·00428 for each ml of 0·1 N solution.

The above methods can only be used for routine determinations on samples which have recently become sour. Where exact determinations are required or where decomposition is advanced, the Government Laboratory Process should be used.

In addition to lactic acid, which is produced from lactose through the intermediate formation of galactose, many volatile products are formed during souring; these include acetic acid, butyric acid, carbon dioxide, alcohol, and ammonia. The Government Laboratory Process allows for appropriate corrections to the non-fatty-solids due to the loss of volatile products.

Preparation of sample

The whole contents of the bottle are turned out into a beaker and whisked for a minute or two with a brush made of fine wire; the inside of the bottle is scraped all over with a wire, some of the milk is poured back, and the contents shaken; this is now emptied into the beaker and again whisked.

Many samples on mixing yield a portion of their fat in a churned condition, which adheres to the wire brush; in these cases a separate estimation of the churned fat should be made.

The Government Laboratory or maceration process (" Somerset House process ")

Dr. James Bell, formerly Principal of the Inland Revenue Laboratory at Somerset House, on being appointed referee under the Sale of Food and Drugs Act, devised this method for the determination of fat and solids-not-fat in milk, and it was later perfected at the Government Laboratory. Two papers discussing the process were subsequently published, one by Thorpe (1905) and the other by Richmond and Miller (1906); the following details of procedure are those employed by Richmond and are essentially those of the Government Laboratory.

About 10 g of milk are weighed into a basin of platinum or aluminium about 3 inches in diameter and a little over 1 inch high, with a flat bottom, provided with a flat-ended glass stirrer. Two drops of a 0·5 per cent solution of phenolphthalein are added, and approximately N/11 strontia solution run in till a faint pink colour appears. The contents are evaporated to a damp paste on the water-bath, when the basin is transferred to a hot-plate, and the paste mixed with the stirrer; at a certain point in the evaporation the paste comes away from the basin and by careful manipulation both basin and stirrer can be obtained practically clean. On further evaporation and stirring, the paste begins to get into a condition in which it can be broken up and rubbed into pieces, and at this stage it is removed from the hot-plate and about 20 ml of methylated ether (specific gravity 0·720), dried with calcium chloride, are added. On gentle rubbing with the stirrer, the solids begin to go to pieces; the stirrer and basin are now scraped with a knife or spatula to bring any small portions of solids adhering to the sides under the ether, and the solids are gently rubbed to a powder. The ether is decanted through a weighed filter 9 cm in diameter, and the solids treated again with ether. The solids at this stage are in a condition in which they can be ground to powder; the ethereal solution is allowed to settle, and the ether decanted through the filter; without any further addition of ether the solids are now ground up to a very fine powder. It is advisable to do this with only a very little ether in the basin, as it is then easy to see the larger portions, which can be ground up one by one. A further addition of ether is made, and the solids ground further; the ether at this stage looks like whitewash, and the solids take some minutes to subside sufficiently to allow of decantation. After about six or eight treatments in this manner the solids are allowed to air-dry, the portions clinging to the stirrer and sides of the basin scraped down, and 5 ml of alcohol and a few drops of water are added; the solids are well mixed with the alcohol, and the basin is placed on the hot-plate and evaporated till the paste begins to go to pieces, when the solids are treated as before. A second treatment with alcohol and a further six to eight extractions with ether are given; the filter gradually becomes partially blocked with the finely-divided solids, but never to such an extent that filtration stops. The solids are air-dried, and then dried in the water-oven to constant weight (0·00428 gram is subtracted for each ml of 0·1 N strontia used). At the Government Laboratory the solids-not-fat are transferred to a weighing-bottle, but Richmond and Miller omit this, since although the solids-not-fat are hygroscopic, no appreciable error is due to this omission. The final weight is known from previous weighings to within a very small amount, and consequently the time of weighing is very short, and not more than a few tenths of a milligram of hygroscopic moisture, are taken up during the weighing. The top of the filter, where fat collects, is cut off and cut into pieces and washed thoroughly with ether; the knife or spatula is wiped on some of the pieces cut off to remove any particles of solids, and the filter containing the pieces cut off is placed in a weighing-bottle and dried in the water-oven to constant weight. The ether is distilled, and the residue of fat weighed; the fat is extracted with petroleum ether, and the small residue insoluble in this solvent subtracted from the weight; this usually consists of phenolphthalein, and its weight may be neglected without appreciable error.

Use of maceration method for fresh and soured milks: (a) Fat

It was shown by Thorpe, and by Richmond in conjunction with Hehner and Bevan, and later with Miller, that the results of determination of fat by the maceration method agree with those obtained by other methods which are admitted to give substantially accurate results; and Simmonds has also obtained results which show that accurate determinations can be made on samples of homogenised milk.

At the Government Laboratory it has been shown that the fat in the sour milk varies from 0·06 per cent more to 0·15 per cent less than in the fresh milk, and averages 0·05 per cent less.

Richmond and Miller found that the results on eighteen samples of sour milk were in very fair agreement with those obtained when fresh by Gottlieb's method; the results varying from 0·10 per cent above to 0·18 per cent below, and averaging 0·03 per cent below.

The difference between the results obtained on the fresh milk and those on the sour milk is partly due to the difficulty of completely re-distributing the fat in the curdled milk. Examination of an apparently well-mixed sample of sour milk with a low-power lens shows the presence of quite large particles of cream, and no amount of whisking with a wire brush appears to reduce the milk to the same homogeneous condition easily obtained with fresh milk.

(b) Solids-not-fat

Richmond and Miller made a number of comparisons of the solids-not-fat by the maceration method with those obtained by the S.P.A. method, and find that invariably the former were higher than the latter. The average difference was 0·20 per cent.

It appears from experiments by Richmond and Miller that the solids-not-fat obtained by the maceration method contain a portion of the sugar as hydrated sugar, but the amount of water of hydration, which averages about 0·1 per cent, is not sufficient to explain the difference between the results.

Another source of error in the maceration method is due to the presence of aldehydes in the ether; milk solids remove the aldehyde completely from ether, and this appears to be due to a condensation of the —COH group with the free amino-groups of the protein. The solids-not-fat obtained by the maceration method are always more acid than the milk, and the aldehyde figure is less, the increase of acid and the decrease in the aldehyde figure being identical within the limits of experimental error. These figures afford data for the estimation of the increase of weight due to the condensation of the aldehyde, and assuming that it is acetaldehyde, the error is almost constantly 0·03 per cent, unless freshly-distilled aldehyde-free ether be used.

Even this addition does not explain the whole of the difference between the maceration and S.P.A. methods, and the marked browning of the residue in the latter method suggests that the remainder of the difference is due to the results obtained by it being too low; this conclusion is strengthened by the fact that evaporation over a large surface, whereby browning of the residue is avoided, gives slightly higher results.

Extraction method for fat and S.N.F. (Macdonald 1944)

The following method is more rapid than that of the Government Laboratory. It is suitable for all milks other than those in which considerable proportions of butyric acid have been formed, and requires about 15 ml for the complete analysis.

Determination of fat and uncorrected S.N.F.

Dry a 150-ml conical flask, *A*, and a plug of non-absorbent cotton wool (*ca.* 0·4 g) for one hour at 98°/100° C, cool in a desiccator, and weigh. Set the plug aside, reweigh the flask, introduce 10·3 ± 0·05 g of the milk, and reweigh. Add 50 ml of *n*-heptane (b.p. 99°/100° C) and fit the flask to a Dean and Stark receiver and condenser. Fill the receiver, which is graduated up to 10 ml and fitted with a tap, with the same solvent. Boil the mixture for two hours on a hot plate, at the end of which time the moisture, volatile acids and alcohol will have collected in the receiver and practically the whole of the fat will be dissolved in the *n*-heptane. Set the receiver aside for the subsequent determination of volatile acids and alcohol. Decant the solution of fat from flask *A* into a previously dried and tared flask *B*, and recover the solvent by distillation. Next, by grinding the remaining solids in flask *A* with 6–8 successive 25 ml portions of light petroleum (b.p. 40°/60° C, dried over $CaCl_2$), which are filtered through the cotton plug fitted in a 4-cm glass funnel, transfer remainder of the fat to flask *B* and rinse the funnel and plug with a little light petroleum, remove the light petroleum on the water-bath, and dry the flask *B* and fat to constant weight at 98°/100° C. Remove any solids adhering to the glass rod used for completing the separation of the fat by wiping with the cotton plug moistened with a few drops of 50 per cent alcohol. Put the plug into flask *A* with the solids-not-fat, and dry at 98°/100° C until constant in weight.

By this process efficient separation of the fat and solids-not-fat is effected. No re-extraction of the separated fat is necessary. Although there is slight browning of the solids, this does not appear to affect the accuracy of the method.

Determination of volatile acids

Add water to the aqueous distillate in the receiver to bring the volume to 10 ml and, after mixing, run the watery layer into a dry test tube. Titrate 5 ml with 0·1 N sodium hydroxide, using 5 drops of 0·5 per cent phenolphthalein solution as indicator. Calculate the result as per cent acetic acid.

This is applicable to the bulk of sour milks encountered, but, as mentioned above, is not suitable for those in which appreciable proportions of butyric acid have developed. Acetic and propionic acids are recovered quantitatively when present in the milk in total proportions up to 1 per cent, whether singly or together. For concentrations of total volatile acid up to 0·2 per cent, the maximum error involved in the ultimate s.n.f. correction by the assumption that all of the acid is acetic would be of the order of 0·1 per cent. With concentrations higher than this, advantage may be taken of the oxidation method of Fyleman (1924) for the determination of the higher member of the series, the lower being obtained by difference.

Determination of alcohol (Snell and Snell, 1937)

Allow a further portion of the aqueous distillate to react at room temperature with a nitric acid solution of dichromate, and measure in a Lovibond Tintometer the resultant decrease in yellow units caused by the reduction of the dichromate. The presence of acetic or propionic acid in concentrations up to 1 per cent does not interfere under the experimental conditions. Pipette 1 ml of the aqueous distillate into a stoppered test-tube graduated at 10 ml. Add 2 ml of a solution of potassium dichromate in nitric acid (0·25 per cent in HNO_3 1 : 2 v/v), mix, and leave for 24 hours at laboratory temperature. Allow a further 1 ml of the aqueous distillate to react with 2 ml of potassium dichromate solution (1·25 per cent in HNO_3 1 : 2 v/v) in a 50-ml graduated flask under the same conditions as above. After the reaction is complete (24 hours) make the solutions up to volume and record the yellow units in a 1 cm cell in a Lovibond Tintometer. By the use of these two concentrations of dichromate, from 0 to 1 mg of alcohol per ml and 1 to 5 mg of alcohol per ml may be estimated respectively, the proportions of alcohol normally encountered being within these limits.

The graphs relating alcohol to yellow units are quite smooth, and pass through the following points—

Potassium dichromate 0·25 *per cent*

Alcohol in mg	0	0·2	0·4	0·6	0·8	1·0
Lovibond yellow units . .	7·0	5·3	4·0	2·9	2·0	1·4

Potassium dichromate 1·25 *per cent*

Alcohol in mg	0	1·0	2·0	3·0	4·0	5·0
Lovibond yellow units . .	7·0	5·3	3·8	2·5	1·5	0·7

Satisfactory recoveries of alcohol, acetic acid and propionic acid were obtained by the above methods from mixtures of the three in various proportions, both from aqueous solution and from fresh milk to which additions had been made.

Determination of ammonia

Conway's (1933) method provides a useful means of determining ammonia in the proportions normally encountered in sour milks.

Pipette 1 ml of N hydrochloric acid into the central chamber of a Conway unit, whose rim has been smeared with vaseline, and introduce 2 ml of milk into the outer chamber. Add 1 ml of a saturated solution of potassium carbonate, quickly replace the lid, and leave the unit for two hours at laboratory temperature. Then by means of a fine-pointed pipette transfer the acid to a 100-ml graduated vessel, rinse the central chamber three or four times with ammonia-free water, and add the washings to the same flask. Make up the mixture to volume, dilute a suitable aliquot portion to 50 ml in a Nessler cylinder, add 2 ml of Nessler reagent, and match the colour disc in a B.D.H. Nessleriser.

Corrections of s.n.f. for decomposition products

Alcohol: For each 184 parts of alcohol add 342 parts.

Ammonia: For each part of ammonia add 5·2 parts.

Volatile acids: For each 60 parts of acetic acid add—

(*a*) Sixty parts to correct for removal of acetic acid from solids during distillation;
(*b*) 25·5 parts to correct for decomposition of lactose during souring.

For each 74 parts of propionic acid add—

(*a*) Seventy-four parts to correct for removal of propionic acid from solids during distillation;
(*b*) 68·5 parts to correct for the decomposition of lactose during souring.

For twenty samples of sour milk examined by this method the following differences from the original milk were found—

fat: — 0·21 to + 0·03,
s.n.f.: — 0·25 to + 0·20

These differences are of the same order as those obtained when the Government Laboratory method is used.

Estimation of fat and s.n.f. by treatment with ammonia

The following method has been found to be of considerable use for routine purposes. Add to the whole sample sufficient of a measured volume of 3 N ammonia to provide a slight excess as judged by the odour. Mix well, but gently, avoiding the formation of an emulsion, and leave overnight at room temperature. As a rule the milk is then in a suitable condition for examination, but with more obstinate samples resort may be made to gentle warming. Measure the volume of the mixture and calculate the percentage by volume of 3 N ammonia. Take the specific gravity of the mixture by means of a lactometer or Sprengel tube and apply a correction of + 0·55 of a lactometer degree for each 1 per cent of 3 N ammonia present. In most samples the fat may be estimated by the Gerber method, but where much butyric acid has developed the Werner-Schmidt process may be employed. Multiply the result thus obtained by the appropriate factor to correct for the dilution of the original sample by the added ammonia. Calculate the s.n.f. by the Richmond formula.

For twenty samples of sour milk examined by this method the following differences from the original milk were found—

fat: $-0{\cdot}30$ to $+0{\cdot}05$
s.n.f.: $-0{\cdot}28$ to $+0{\cdot}04$

Determination of decomposition products (Government Laboratory method): (i) Determination of alcohol

About 75 g are weighed into a 300 ml flask for the estimation of alcohol, and half neutralised with 0·5 N soda solution; about half the volume is distilled and collected in a small flask, and neutralised with 0·5 N soda, using litmus paper as indicator. From this, 25 ml are distilled, and the density taken at 60° F by a Sprengel tube. From the density the percentage of alcohol is calculated by Table 21.1 (p. 444).

At the Government Laboratory the percentage of alcohol is deduced by multiplying the difference between 1 and the specific gravity of the distillate by 1,000 × 1·16, and this gives it as percentage of proof spirit; as proof spirit contains 49·5 per cent alcohol by weight, it is evident that the factor 1,000 × 0·572 will give the percentage of alcohol by weight.

The total acidity to litmus paper may be calculated as lactic acid; from this an amount equivalent to the volatile acids is subtracted.

(ii) Determination of volatile acids

The method used at the Government Laboratory for the determination of volatile acids is as follows—

Ten g of milk are neutralised to the extent of one-half the total acidity with 0·1 N NaOH, and a little phenolphthalein added. The mixture is then evaporated to dryness on a water-bath with frequent stirring, and, after treatment with 20 ml of boiling distilled water so as to break up and detach the milk solids thoroughly from the capsule, a further addition of 0·1 N NaOH is made, until the neutral point is reached. The difference between the original acidity of the milk and that of the evaporated portion is regarded as acetic acid. The number of ml of soda shown, when multiplied by 0·06, will give the percentage of acetic acid.

Example

Acidity of original milk = 11·6 ml 0·1 N NaOH
Acidity of evaporated portion = 9·2 ,,
Difference = 2·4 ,,

or, $2{\cdot}4 \times 0{\cdot}006 \times 10 = 0{\cdot}144$ per cent of acetic acid.

Richmond and Miller have shown that this method is accurate only when the volatile acidity lies between 0·1 and 0·2 per cent of acetic acid, as is the case in the majority of sour milks.

It sometimes happens that a considerable quantity of butyric acid is formed, and then it is preferable to employ the following modification of Duclaux's method—

Add to the milk from which the alcohol has been distilled a quantity of acid exactly equal to the soda used for half-neutralising, and distil this to a small

Table 21.1—Alcohol table

(Calculated by Richmond from those of Thorpe and the U.S. Bureau of Standards)

Specific gravity at $\frac{60^\circ\text{ F}}{60^\circ\text{ F}}$	Alcohol per cent by weight	Specific gravity at $\frac{60^\circ\text{ F}}{60^\circ\text{ F}}$	Alcohol per cent by weight	Specific gravity at $\frac{60^\circ\text{ F}}{60^\circ\text{ F}}$	Alcohol per cent by weight
0·9999	0·05	0·9969	1·66	0·9939	3·37
8	0·11	8	1·72	8	3·43
7	0·16	7	1·77	7	3·48
6	0·21	6	1·83	6	3·54
5	0·26	5	1·88	5	3·60
4	0·32	4	1·95	4	3·66
3	0·37	3	2·00	3	3·72
2	0·42	2	2·06	2	3·78
1	0·47	1	2·11	1	3·84
0	0·53	0	2·17	0	3·90
0·9989	0·58	0·9959	2·22	0·9929	3·96
8	0·64	8	2·28	8	4·02
7	0·69	7	2·33	7	4·08
6	0·75	6	2·39	6	4·14
5	0·80	5	2·44	5	4·20
4	0·85	4	2·51	4	4·26
3	0·90	3	2·56	3	4·32
2	0·96	2	2·62	2	4·39
1	1·01	1	2·67	1	4·45
0	1·07	0	2·73	0	4·51
0·9979	1·12	0·9949	2·79	0·9919	4·57
8	1·18	8	2·85	8	4·63
7	1·23	7	2·90	7	4·69
6	1·29	6	2·96	6	4·76
5	1·34	5	3·02	5	4·82
4	1·39	4	3·08	4	4·88
3	1·44	3	3·13	3	4·94
2	1·50	2	3·19	2	5·01
1	1·55	1	3·25	1	5·07
0	1·61	0	3·31	0	5·13

bulk; water is then added in successive quantities of about 25 ml, and distilled off till the distillate is practically neutral.

The mixed distillates, neutralised to phenolphthalein are made up to 250 ml, and an aliquot portion, preferably taking 20 to 30 ml 0·1 N alkali, taken for fractional distillation; to this is added a quantity of N sulphuric acid, very slightly greater than is necessary to neutralise the soda used, and the whole made up to 100 ml. Nine successive fractions, each about one-tenth of the total volume, are distilled and titrated separately with 0·1 N strontia; 25 ml of water are added to the residue, and a further 25 ml distilled and titrated, and this treatment may be repeated till no more volatile acid comes over. From the last titration the total quantity of volatile acid is obtained: this is always slightly less than the total acidity of the first distillate, due, no doubt, to the presence of a little lactic acid. As much as possible of the first distillate is made up to a convenient bulk, after adding a quantity of N sulphuric acid in slight excess of that required to neutralise the soda, and exactly one-third is distilled; this is made up to a convenient bulk, and exactly one-third is again distilled. The last distillate is made up to 100 ml, and nine fractions of 10 ml each are distilled and titrated separately. The residues in the flask and condenser are washed out and titrated to obtain the total quantity.

Richmond has shown that the rate of distillation of the lower fatty acids varies slightly with the concentration, and has calculated tables to allow for these; they have been deduced from the mean of a number of distillations, and apply to strengths of acid such that 100 ml of solution take about 10 to 50 ml of 0·1 N acid.

In Table 21.2, the column marked x is the percentage of solution or ml per 100 ml distilled; the columns marked y give the molecular percentage of acid passing over, and that marked $\frac{\Delta y}{\Delta x}$ the factor to be used to multiply a small excess or deficiency in the volume distilled to correct for corresponding percentage of acid. Thus, if 30·15 ml out of 100 ml were distilled and $\frac{\Delta y}{\Delta x} = 0{\cdot}77$, 0·12 ($= 0{\cdot}15 \times 0{\cdot}77$) must be added to the percentage of acid distilled given in the Table for 30 ml to find the theoretical amount distilled for 30·15 ml.

For each fraction, calculate from the table the proportion of each of the acids which would distil, and calculate the ratios of butyric to acetic, and butyric to propionic acid, which correspond to the proportion actually distilled. The ratios from first and last fractions are liable to be slightly erroneous, the first because a small amount of a very volatile acid, such as carbonic, would appear in this fraction, and the last because experimental error is greatly magnified, so that these should carry only half weight; but usually the other ratios are practically constant for either butyric and acetic acids, or butyric and propionic acids.

The distillation of the third of a third is undertaken in order to decide definitely the composition of the mixture. From a number of experiments it has been found that the proportions which would distil, when one-third of one-third is collected, are—

For butyric acid	0·325
,, propionic acid	0·163
,, acetic acid	0·063

Thus, a mixture of butyric and propionic acids in equal proportions should yield approximately a 2 : 1 mixture in the distillate, while a mixture of butyric and acetic in equal proportions would yield a 5 : 1 mixture. It is even possible o deduce the relative proportions of a mixture of the three acids.

Table 21.2—Distillation of volatile acids

ACETIC

x	$\frac{\Delta y}{\Delta x}$	y				
		0·01N	0·02N	0·03N	0·04N	0·05N
10	0·71	6·45	6·55	6·65	6·75	6·8
20	0·74	13·55	13·8	13·9	14·0	14·1
30	0·77	20·9	21·25	21·35	21·5	21·6
40	0·80	28·85	29·2	29·4	29·55	29·65
50	0·85	37·2	37·55	37·65	37·8	37·9
60	0·91	46·15	46·5	46·6	46·75	46·85
70	1·00	55·65	56·0	56·1	56·25	56·35
80	1·16	66·25	66·6	66·7	66·85	66·85
90	1·41	78·5	79·1	79·2	79·35	79·45

PROPIONIC

10	1·22	12·35	12·4	12·4	12·4	12·45
20	1·18	23·9	24·1	24·2	24·4	24·5
30	1·14	35·7	35·8	35·9	36·05	36·1
40	1·10	46·8	46·9	47·0	47·1	47·2
50	1·05	57·2	57·4	57·6	57·7	57·75
60	1·00	67·3	67·5	67·7	67·9	68·0
70	0·93	77·1	77·2	77·3	77·4	77·45
80	0·84	85·6	85·8	86·0	86·2	86·3
90	0·71	93·8	93·8	93·8	93·85	93·85

BUTYRIC

10	1·80	19·9	20·15	20·4	20·5	20·55
20	1·60	36·2	36·6	37·0	37·2	37·1
30	1·40	51·1	51·5	51·9	52·1	52·2
40	1·20	63·75	64·15	64·55	64·75	64·9
50	1·00	74·5	74·9	75·3	75·5	75·6
60	0·80	83·3	83·7	84·1	84·3	84·4
70	0·60	90·35	90·75	91·1	91·3	91·4
80	0·40	95·25	95·65	95·9	96·1	96·2
90	0·20	98·85	98·95	99·05	99·1	99·15

Methods of calculation

If two acids only are present, the equation for calculating the results is $aA + bB = R$, where

$a =$ fraction of acid (A) from Table 21.2
$b =$,, ,, (B) ,, ,, ,,
$R =$,, ,, actually distilled
$A =$,, ,, (A) present
$B =$,, ,, (B) ,,

This simplifies to $A = \frac{R - b}{a - b}$, and nine values are obtained of the ratio of the nine distillations $(B = 1 - A)$. If three acids are present, the equation becomes $aA + bB + pP = R$, which simplifies to $B + P\frac{p - a}{b - a} = \frac{R - a}{b - a}$, which gives nine simultaneous equations to be solved; and the problem is how to obtain mean values for P and B (and also for A, which is $1 - P - B$) with the least labour and greatest accuracy.

The tables below give the difference between each pair of acids for each 10 ml distilled out of 100 ml, together with difference factors for calculating small differences in volume. These may be used for calculating the values of $\frac{p - a}{b - a}$ and $\frac{R - a}{b - a}$.

To facilitate calculation still more, a table of the values of $\frac{p - a}{b - a}$ has also been calculated. The values of $\frac{p - a}{b - a}$ and $\frac{R - a}{b - a}$ are then tabulated.

The values of $\frac{p - a}{b - a}$ and $\frac{R - a}{b - a}$ for 90 ml are subtracted from those for 10 ml (1); those for 80 ml subtracted from those for 20 ml (2); those for 70 ml from those for 30 ml (3); those for 60 ml from those for 40 ml (4). Then the values for 50 ml are subtracted successively from those for 40, 30, and 20, and the sum of these last three called (5). The sum of 2 (1) + 3 (2) + 2 (3) + (4) + $\frac{2}{3}$ (5) for $\frac{R - a}{b - a}$ divided by the similar sum for $\frac{P - a}{b - a}$ will give the ratio of propionic to total acid. Next, *nine* values of B (= ratio of butyric acid) are obtained from the equation $B = \frac{R - a}{b - a} - P\frac{p - a}{b - a}$, and a probable value of B is best obtained by summing the nine values of $\Sigma\frac{R - a}{b - a}$ and $P\frac{p - a}{b - a}$ and taking $\frac{1}{9}$ the difference of the sums. The ratio of acetic acid is $1 - B - P$. A 10-inch slide rule is just adequate for the calculation, or four-figure logarithms may be used.

Table 21.3—Difference tables

Propionic acid − acetic acid (p − a)

Strength as normal . .		0·01	0·02	0·03	0·04	0·05
Per cent volume distilled	$\frac{\Delta y}{\Delta x}$	Difference of per cent acid distilled				
10	0·51	5·9	5·85	5·75	5·65	5·65
20	0·44	10·35	10·3	10·3	10·4	10·4
30	0·37	14·8	14·55	14·55	14·55	14·5
40	0·30	17·95	17·7	17·6	17·55	17·55
50	0·20	20·0	19·85	19·95	19·9	19·85
60	0·09	21·15	21·0	21·1	21·15	21·15
70	−0·07	21·45	21·2	21·2	21·15	21·1
80	−0·32	19·35	19·2	19·3	19·35	19·35
90	−0·70	15·05	14·7	14·6	14·5	14·4

Butyric acid − acetic acid (b − a)

Strength as normal . .		0·01	0·02	0·03	0·04	0·05
Per cent volume distilled	$\frac{\Delta y}{\Delta x}$	Difference of per cent acid distilled				
10	1·09	13·45	13·6	13·75	13·75	13·75
20	0·86	22·65	22·8	23·1	23·2	23·2
30	0·63	30·2	30·25	30·55	30·6	30·6
40	0·40	34·9	34·95	35·15	35·35	35·4
50	0·15	37·3	37·35	37·6	37·7	37·7
60	−0·11	37·15	37·2	37·5	37·55	37·55
70	−0·40	34·9	34·75	35·0	35·1	35·05
80	−0·76	29·0	29·05	29·2	29·25	29·25
90	−1·21	20·1	19·85	19·85	19·75	19·7

$$\frac{\textit{Propionic acid} - \textit{acetic acid}}{\textit{Butyric acid} - \textit{acetic acid}} \left(\frac{p-a}{b-a}\right)$$

Strength as normal . .		0·01	0·02	0·03	0·04	0·05
Per cent volume distilled	$\frac{\Delta y}{\Delta x}$	Fractional values				
10	+0·0042	0·4377	0·4302	0·4182	0·4109	0·4109
20	+0·0023	0·4568	0·4517	0·4459	0·4482	0·4482
30	+0·0015	0·4901	0·4810	0·4763	0·4756	0·4740
40	+0·0028	0·5144	0·5065	0·5007	0·4964	0·4957
50	+0·0031	0·5362	0·5315	0·5293	0·5279	0·5266
60	+0·0038	0·5693	0·5645	0·5627	0·5631	0·5631
70	+0·0065	0·6181	0·6101	0·6056	0·6026	0·6018
80	+0·0063	0·6673	0·6610	0·6610	0·6600	0·6627
90	+0·0103	0·7487	0·7404	0·7355	0·7341	0·7308
Σ		−1·663	−1·660	−1·700	−1·706	−1·715

The examples worked out below will show the manner in which the tables can be used to facilitate the working out of results. The working is all put down, though, naturally, much would be done mentally in practice.

Mixture of 20 ml N acetic acid + 20 ml N butyric acid made up to 100 ml—

Table 21.4—Mixture of acetic and butyric acids

Per cent volume distilled	R	R — acetic*	Butyric — acetic†	$\frac{R-a}{b-a}$
10·0	13·6	6·85	13·75	0·498
20·0	25·6	11·6	23·2	0·500
30·0	36·8	15·3	30·6	0·500
40·1	47·3	17·67	35·39	0·499
50·0	56·6	18·8	37·7	0·499
60·0	65·5	18·75	37·55	0·499
70·0	73·8	17·55	35·1	0·500
80·0	81·45	14·6	29·25	0·499
90·0	89·2	9·85	19·75	0·499

* From Table 21.2 † From Table 21.3

$$\text{Mean value of } \frac{\text{butyric}}{\text{total}} = 0{\cdot}499.$$

If calculated as a mixture of acetic, propionic, and butyric acids, the value of (1) is — 0·001, (2) + 0·001, (3) 0·000, (4) 0·000, (5) 0·002. It is evident that the value of

$$\frac{\Sigma \dfrac{R-a}{b-a}}{\Sigma \dfrac{p-a}{b-a}} \text{ will be practically nothing } \left(= \frac{0{\cdot}0023}{-1{\cdot}706} = -0{\cdot}001\right).$$

The mean values will be—

Butyric acid	0·500
Propionic acid	—0·001
Acetic acid	0·501

These ratios are molecular ratios, and must be multiplied by the molecular weights of the acids (88, 74, and 60 respectively) to work out the percentage ratios.

A distillation of propionic acid was made; this was estimated to contain, from the results of distillation in fractions, 2 per cent of acetic acid and 2·6 per cent of butyric acid as molecular ratios. A solution of 0·0394 N was made, and 100 ml were distilled.

Table 21.5—Mixture of three acids

Per cent volume distilled	R	$R - a$*	$b - a$†	$\frac{R - a}{b - a}$	$\frac{p - a\dagger}{b - a}$
9·9	12·55	5·87	13·64	0·4303	0·4105
19·95	24·45	10·49	23·16	0·4530	0·4481
29·95	36·0	14·54	30·57	0·4756	0·4755
40·0	47·1	17·55	35·36	0·4963	0·4964
49·95	57·95	20·19	37·69	0·5356	0·5277
60·0	67·95	21·20	37·55	0·5636	0·5631
70·15	77·75	21·35	35·04	0·6091	0·6036
80·15	86·4	19·38	29·14	0·6851	0·6609
90·15	94·6	15·04	19·57	0·7086	0·7367

* Table 21.2 † Table 21.3

Table 21.6—Calculation of $\frac{R - a}{b - a}$.

$$\begin{array}{rl}
(1) & = 0{\cdot}4303 - 0{\cdot}7086 = -0{\cdot}2783 \quad \times 2 = -0{\cdot}557 \\
(2) & = 0{\cdot}4530 - 0{\cdot}6651 = -0{\cdot}2121 \quad \times 3 = -0{\cdot}636 \\
(3) & = 0{\cdot}4756 - 0{\cdot}6091 = -0{\cdot}1335 \quad \times 2 = -0{\cdot}267 \\
(4) & = 0{\cdot}4963 - 0{\cdot}5636 = -0{\cdot}0673 \quad \times 1 = -0{\cdot}067 \\
(5) & \left.\begin{array}{l} = 0{\cdot}4530 - 0{\cdot}5356 = -0{\cdot}0826 \\ = 0{\cdot}4756 - 0{\cdot}5356 = -0{\cdot}0600 \\ = 0{\cdot}4963 - 0{\cdot}5356 = -0{\cdot}0393 \end{array}\right\} \times \tfrac{2}{3} = \underline{-0{\cdot}121} \\
 & \hfill \underline{-1{\cdot}648}
\end{array}$$

Similarly, $\Sigma\frac{p - a}{b - a}$ (it can also be worked out from the sum and the $\frac{\Delta y}{\Delta x}$ column) is $-1{\cdot}717$. Hence

$$\frac{1{\cdot}648}{1{\cdot}717} = 0{\cdot}960$$

The total of the nine values of $\frac{R - a}{b - a} = 4{\cdot}9372$, and of the nine values of $\frac{p - a}{b - a} = 4{\cdot}9195 \times 0{\cdot}96 = 4{\cdot}7233$, leaving 0·214, which divided by 9 = 0·024.

The composition of the acid is, therefore, in molecular ratios—

Propionic acid	0·960 or	95·9 per cent by weight.
Butyric acid	0·024 ,,	2·8 ,, ,,
Acetic acid	0·016 ,,	1·3 ,, ,,

(iii) Determination of ammonia

Certain changes take place in the proteins in sour milk, and at the Government Laboratory to estimate ammonia 2 g of the milk are made up to 100 ml with distilled water, and filtered to a clear solution. Ten ml of the filtrate, increased to 50 ml by the addition of distilled water, are nesslerised against NH_4Cl solution, equivalent to 0·01 mg NH_3 in each ml. As the Nessler colour produced in the presence of milk differs somewhat from that of pure saline ammonia, the blank experiment is carried out with the addition of 10 ml of the filtrate from 2 g of new milk slightly acidified, and diluted to the same extent as the sour milk. The quantity of test ammonia solution required varies from 0·5 to 4·0 ml. With milk containing ammonia equal to 2·6 ml of the test solution, the ammonia is calculated as follows—

$$0{\cdot}01 \times 2{\cdot}6 \times 0{\cdot}5 = 0{\cdot}013 \text{ per cent ammonia.}$$

It is evident that any other degree of dilution may be adopted according to circumstances, or to the proportion of ammonia which may be indicated in the milk.

(iv) Determination of carbon dioxide

Carbonic acid can only be estimated in koumiss or kephir contained in a corked bottle. The worm of a champagne tap is carefully turned off to leave a perfectly smooth stem; the tap is also carefully reground to make sure that it fits. A drying and absorbing apparatus is fitted up, consisting of (*a*) a U-tube containing pumice and sulphuric acid, (*b*) a U-tube containing soda lime immersed in a beaker of cold water, and (*c*) a U-tube filled half with soda-lime and half with calcium chloride. These are connected in the order named, and the end of (*a*) is connected by a short piece of rubber tubing to the champagne tap.

(*b*) and (*c*) are weighed, and the tap (closed) carefully forced through the cork of the bottle; the tap is opened slightly and the carbon dioxide allowed slowly to escape; when the escape of gas becomes slack, the bottle may be warmed slightly by placing it in warm water, and shaken to promote further escape. When no more gas comes off, the tap is disconnected, a soda-lime tube substituted, and a current of air drawn through the apparatus.

(*b*) and (*c*) are dried, cooled, and weighed again; the increase represents the amount of carbon dioxide which has escaped from the bottle. The total contents of the bottle are now weighed and the percentage is calculated.

There remains still a little carbon dioxide dissolved; this can be determined by titrating a weighed amount with 0·1 N baryta water, using phenolphthalein as indicator; the difference between the acidity thus determined and that determined as previously described will represent, without great error, the carbon dioxide (1 ml 0·1 N alkali = 0·0022 g CO_2). This should be added to the amount determined by absorption.

Corrections for solids-not-fat in sour milk

When it is desired to ascertain from the analysis of a sour milk the original composition of the sample, the following constituents should be determined: fat and solids-not-fat by the maceration method; alcohol, ammonia, acidity, and aldehyde figure of the milk—i.e. volatile acidity by the Government

Laboratory method, and aldehyde figure of the neutralised solution obtained in this method. If the volatile acidity is high, the proportions of butyric, propionic, and acetic acids should be determined by Duclaux's method. The solids-not-fat should be mixed with 10 ml of hot water, and the acidity or alkalinity and aldehyde figure determined.

The following corrections should be made—

Alcohol correction

(1) For each 184 parts of alcohol add on 342 parts, or the difference between 1 and the specific gravity of the distillate multiplied by 977 and by the weight of the distillate (or volume in ml), and divided by the weight of milk taken.

Volatile acid corrections

(2) For each 60 parts of acetic acid add on 25·5 parts.
(3) For each 74 parts of propionic acid add on 68·5 parts.
(4) For each 88 parts of butyric acid add on 87·5 parts.

Ammonia correction

(5) For each part of ammonia add on 5·2 parts.

Lactic acid correction

(6) For each part of lactic acid subtract 0·05 part.

Aldehyde correction

(7) For each degree of difference between the aldehyde figure of the neutralised solution obtained in the volatile acid estimation and that of the solids-not-fat, subtract 0·0026 per cent.

Amino-acid correction

(8) For each degree of aldehyde figure of the neutralised solution obtained in the volatile acid determination above 20°, subtract 0·0018 per cent. (This is rarely required.)

Correction for loss of butyric acid

(9) Subtract the acidity of the solids-not-fat from the difference between the aldehyde figures of the volatile acid solution and of the solids-not-fat; multiply the figure thus obtained by 0·0088, and add it to the solids-not-fat.

By this system of corrections Richmond and Miller found that the solids-not-fat deduced from the analysis of sour milks varied from 0·32 per cent above the solids-not-fat in the original milk to 0·20 per cent below, averaging 0·06 per cent above. The determinations on the original milks were made by subtracting the fat by Gottlieb's method from the total solids by evaporation. With milks in which no very large amount of volatile acid was developed the figures were + 0·17, — 0·19, and + 0·07 respectively. At the Government Laboratory, only corrections Nos. 1, 2, and 5, and, if necessary, a correction of 92 parts for each 88 parts of butyric acid are made. Richmond, however, believed that the additional corrections give more exact results, though the difference due to neglecting them is small. The whole system of corrections is based on a long investigation made at the Government Laboratory, which has established that, if milk is adulterated with added water, the percentage added can be deduced from an analysis of the sour milk, and that the figure

Table 21.7—Analyses of sour milk

No.	Fat (original)	Fat (sour)	Difference	Solids-not-fat (original)	Solids-not-fat (sour)	Alcohol	Correction	Butyric acid	Propionic acid	Acetic acid	Volatile acid (correction)	Ammonia	Correction	Aldehyde (correction)	Loss of butyric acid	Lactic acid	Correction	Solids-not-fat, Government Laboratory	Solids-not-fat R. and M.	Age (days)
1	2·77	2·78	+0·01	8·29	8·00	0·165	0·32	—	—	—	—	0·014	0·08	−0·03	—	1·38	−0·07	8·40	8·30	246
2	3·66	3·62	−0·04	8·95	8·90	0·021	0·04	—	—	0·136	0·06	0·009	0·05	−0·03	—	0·94	−0·05	9·05	8·97	31
3	3·66	3·53	−0·13	8·95	9·03	0·021	0·04	—	—	0·133	0·06	0·009	0·05	−0·03	—	0·88	−0·05	9·18	9·10	31
4	4·03	4·12	+0·09	8·83	8·68	0·050	0·10	—	—	0·170	0·07	0·009	0·05	−0·03	—	0·83	−0·04	8·90	8·83	46
5	3·72	3·73	+0·01	8·77	8·85	0·071	0·14	—	—	0·116	0·05	0·002	0·01	−0·03	—	1·16	−0·06	9·05	8·96	49
6	4·06	4·02	−0·04	8·86	7·14	0·271	0·53	0·820	—	0·187	0·90	0·004	0·02	−0·03	0·11	0·25	−0·01	8·59	8·66	49
7	3·92 M3·89	4·02	+0·10 +0·13	8·74 9·04	8·72	0·082	0·16	—	—	0·171	0·07	0·001	—	−0·03	—	1·23	−0·06	8·95	8·86	37
8	3·97	3·81	−0·16	8·77	7·74	0·180	0·35	0·385	0·360	—	0·66	trace	—	−0·03	0·10	0·37	−0·02	8·75	8·80	37
9	3·84	3·92	+0·08	8·82	8·33	0·366	0·71	—	—	0·078	0·03	0·004	0·02	−0·02	—	0·93	−0·05	9·09	9·02	34
10	3·90 M3·86	3·88	−0·02 +0·02	8·90 9·05	7·69	0·092	0·18	0·658	0·236	0·095	0·91	0·012	0·06	−0·02	0·06	0·29	−0·01	8·84	8·87	31
11	3·61	3·64	+0·03	8·84	8·76	0·075	0·15	—	—	0·138	0·06	0·003	0·01	−0·01	—	0·95	−0·05	8·98	8·92	57
12	3·82	3·90	+0·08	8·88	7·21	0·258	0·50	1·086	—	0·605	1·34	0·016	0·09	−0·01	0·10	0·66	−0·03	9·14	9·20	43
13	3·71	3·53	−0·18	8·79	8·51	0·016	0·03	—	—	0·162	0·07	0·013	0·07	−0·03	—	1·01	−0·05	8·68	8·60	55
14	3·48	3·36	−0·12	8·81	7·55	0·015	0·03	0·946	—	0·658	1·22	0·002	0·01	−0·03	0·11	0·53	−0·03	8·81	8·86	40
15	3·57	3·44	−0·13	8·84	8·81	0·032	0·06	—	—	0·108	0·05	0·004	0·02	−0·02	—	0·91	−0·05	8·94	8·87	36
16	2·63	2·54	−0·09	6·50	6·49	0·030	0·06	—	—	0·108	0·05	0·006	0·03	−0·01	—	0·88	−0·04	6·63	6·58	36
17	3·63	3·57	−0·06	8·71	8·74	0·031	0·06	—	—	0·114	0·05	0·024	0·12	−0·01	—	0·97	−0·05	8·97	8·91	33
18	2·70	2·69	−0·01	6·47	6·35	0·086	0·17	—	—	0·084	0·04	0·019	0·10	−0·01	—	0·90	−0·05	6·66	6·60	33

Nos. 2 and 3 were from the same bulk of milk and taken at the same time.
" 7 and 8 " " " different times.
" 9 and 12 " " " "
No. 16 was a mixture of 73·6 parts of 15 and 26·4 parts of water. Water calculated from sour milk solids-not-fat, 25·7 per cent.
No. 18 " 74·3 " 17 and 27·7 " " " " " 25·9 "
The determinations marked M were obtained on the fresh milk by the maceration method.

thus obtained does not differ by more than 3 per cent, and usually by much less, from that estimated by the analysis of the fresh milk.

Richmond and Miller submitted the Government Laboratory method to a critical examination, and generally endorsed the above conclusions.

Table 21.7 gives the results of the analysis of 18 samples of sour milk, corrected according to the foregoing scheme.

Determination of other constituents of sour milk

Ash should be determined on a separate 10 g portion of the whisked sample.

Lactose and cane sugar, if the latter is present, are almost impossible to determine, due to the partial decomposition of these constituents, and they are usually neglected or estimated by difference.

Total acidity and the aldehyde figure are determined as described for fresh milk, except that in the latter determination it is necessary to add a larger quantity of formaldehyde solution on account of the great dilution by the large volume of alkali required for neutralisation.

The freezing point. Evans and Macdonald have described methods for the determination of the original freezing point of sour milk. Details of the methods are given on pp. 141 and 326.

CHAPTER 22

THE ANALYSIS OF MILK PRODUCTS OTHER THAN BUTTER AND CHEESE

For the analysis of milk products, the methods already described can generally be used. The following notes will show where it is advisable to depart from them or to employ modifications of the usual processes.

Homogenised and/or sterilised milk

Owing to the reduced size of the fat globules the cream does not rise, and some methods depending on the abstraction of the fat from the milk (i.e. the Adams process) are inapplicable in that they give low results. Fat should be extracted by the Röse-Gottlieb, the Gerber, or the Leffmann-Beam process; in the two latter processes it is advisable to whirl again and read the butyrometer tubes a second or a third time in order to ascertain that the maximum fat reading has been obtained.

Skimmed milk

Accurate determination of the fat is also more difficult with this product. The special Gerber tubes reading up to only 4 or 5 per cent should be used for greater accuracy, and re-whirled to obtain the maximum reading.

THE ANALYSIS OF CREAM AND CLOTTED CREAM

Determination of total solids

A porcelain milk dish containing some ignited sand and a glass rod is tared, and about 2 g of the cream are added and accurately weighed. The cream is stirred into the sand with the aid of a little hot water and the glass rod. The contents of the dish are then evaporated to dryness on the water-bath, care being taken to stir the sand occasionally with the glass rod to keep the mass friable. The dish is finally dried to constant weight (2 to 3 hours) in an oven maintained at 98° to 100° C.

Ash should be determined on a separate portion of the sample, and at least 5 g used.

Determination of fat

For accurate work the Röse-Gottlieb process should be used. One to 2 g of cream are weighed into the tube, diluted with water to 10 ml, and then the process carried out as described for milk.

Routine determinations of fat in cream are usually carried out by the Gerber or Leffmann-Beam processes, using the technique described in the special paragraphs on cream previously given under these processes (pp. 350 and 356).

An indirect estimation of the percentage of fat may be made from the total solids, and vice versa. For this purpose it is assumed that the proportion of solids-not-fat to water in milk is constant, an assumption which causes no appreciable error in cream analysis. It is found that on the average 100 parts of water contain 10·2 parts of solids-not-fat.

The following formulae will express this relation—

Let T = total solids, F = fat, and S = solids-not-fat.

$$\text{Then} \quad F = 1{\cdot}102T - 10{\cdot}2\ ; \quad S = 10{\cdot}1 - 0{\cdot}1T, \text{ or } \frac{10 - 0{\cdot}1\,F}{1{\cdot}08}.$$

The following table may be used—

Table 22.1—Ratio of fat to total solids in cream (per cent)

Total solids	Fat	Solids-not-fat	Total solids	Fat	Solids-not-fat
60	55·9	4·1	44	38·3	5·7
59	54·8	4·2	43	37·2	5·8
58	53·7	4·3	42	36·1	5·9
57	52·6	4·4	41	35·0	6·0
56	51·5	4·5	40	33·9	6·1
55	50·4	4·6	39	32·8	6·2
54	49·3	4·7	38	31·7	6·3
53	48·2	4·8	37	30·6	6·4
52	47·1	4·9	36	29·5	6·5
51	46·0	5·0	35	28·4	6·6
50	44·9	5·1	34	27·3	6·7
49	43·8	5·2	33	26·2	6·8
48	42·7	5·3	32	25·1	6·9
47	41·6	5·4	31	24·0	7·0
46	40·5	5·5	30	22·9	7·1
45	39·4	5·6	29	21·8	7·2

Should it be necessary to determine the nature of the fat in the cream, it may be separated for analysis by a modification of the Röse-Gottlieb process, carried out in a separating-funnel and employing relatively large amounts of the sample and the reagents. After carefully removing the solvents from the fat, the latter may be examined by the methods described for the differentiation of butter from margarine, etc.

Total nitrogen may be determined by the Kjeldahl method, although the acid digestion may be unduly prolonged because of the difficulty of destroying the large amount of fat present. For this reason it is sometimes preferable to evaporate the cream to dryness in the Kjeldahl flask, and then to extract the bulk of the fat with ether before proceeding with the acid digestion.

Lactose. Should this determination be necessary, it can be carried out by the methods already described for milk and condensed milk. A convenient dilution to work with is 1 part by weight of cream made to 2 parts with water.

Determination of preservatives

Generally speaking, the methods applicable to milk are also applicable to cream.

Formaldehyde. The sample should be diluted with a sample of pure milk and the tests carried out as for milk.

Determination of borates. A direct test with turmeric-paper on the acidified cream may fail to give a reaction in the presence of borates, due to the fat protecting the turmeric-paper from the borate solution. It is advisable, therefore, to render a few grams of the cream alkaline to phenolphthalein with either lime-water or caustic soda solution, evaporate to dryness, char, make slightly acid with dilute hydrochloric acid, and test this solution with turmeric-paper.

The following process for the determination of boric acid is the modification of Thomson's method which is used in the Government Laboratory (Robertson, 1923) and is particularly suitable for cream and other foods containing a large proportion of fat.

A suitable quantity (10 to 20 g) of the sample is weighed into a platinum dish and neutralised with normal caustic soda solution to phenolphthalein and then 2 ml excess added. The contents of the dish are evaporated on a steam-bath, stirring with a glass rod until nearly free from moisture.

Extract the residue twice with ether, and filter the ether extracts through paper into a small separating-funnel. Wash the platinum dish and the filter-paper with ether until free from fat. Wipe adhering solid from the glass rod with the filter-paper, place the paper in the platinum dish, and wash the rod with water into the dish. Wash the ethereal extract first with 5 ml of N sodium hydroxide solution and then with 5 to 6 ml of water, adding the washings to the platinum dish. Add sodium hydroxide solution if necessary, until not less than 2 ml of N sodium hydroxide solution have been added, including that used for original neutralisation, for each gram of dry solid in the sample. Heat the platinum basin gradually to drive off the ether without loss by spurting, evaporate to dryness, and ignite over a low Argand burner. Cover the dish with a platinum cover and continue heating until all organic matter is thoroughly charred. Cool, and wash the charred residue with 10 ml of water, filtering the solution into a 100-ml flask. Wash the dish and paper three times with 5 ml of water. Return the paper to the dish, dry, and ignite carefully over an Argand burner until all the carbon is burned.

Dissolve the ash in dilute hydrochloric acid, pour the solution through the filter funnel into the 100-ml flask, and wash the funnel. Add hydrochloric acid carefully until no precipitate remains. Place the flask on a steam-bath and shake until there is no more effervescence of carbonic acid. Cool. Add 5 ml of N calcium chloride solution, 5 drops of phenolphthalein solution, and N sodium hydroxide solution until a slight permanent precipitate is formed. then add 0·1 N sodium hydroxide solution, drop by drop, with constant shaking, until there is a distinct, but faint, permanent pink colour. Make up to 100 ml. Shake thoroughly, filter off 75 ml and catch any filtrate in excess of the 75 ml in the 100-ml flask (*vide infra*).

Transfer the filtered solution to a 400-ml hard glass beaker. Add 1 drop of methyl orange indicator and 0·1 N sulphuric acid until the liquid is just acid to methyl orange. Cover with a clock-glass and boil for 10 minutes to expel carbonic acid; cool quickly; wash the cover into the beaker with water; add 10 drops of phenolphthalein, and bring the liquid to neutral point to methyl orange. (One drop of 0·1 N acid or alkali is usually sufficient.) Add 0·5 g of mannitol, and titrate with 0·1 N sodium hydroxide solution to a pink colour. Add another portion of mannitol, and if the pink colour is not discharged note the number of millilitres added. If the colour is discharged, add more sodium hydroxide solution until further addition of mannitol fails to discharge the colour. A blank obtained by using the same quantities of sodium hydroxide solution and other reagents as in the test experiment is subtracted from the number of millilitres used. Calculate the number of millilitres required for the 100 ml of liquid, representing the boric acid in the quantity of the sample weighed out. Each ml of 0·1 N sodium hydroxide solution is equivalent to 0·0062 g of boric acid (H_3BO_3).

The residue on the filter and in the 100-ml flask is dissolved in hydrochloric acid and collected in the 100-ml flask. Phenolphthalein and 1 ml of N calcium chloride are added, and the process of precipitation of phosphate, filtration and titration is repeated as described above. The difference between this titration figure and one-fourth of the original titration figure is negligible if the phosphate has been carefully precipitated. If, however, the difference is appreciable, it should be added to the original titration.

Thickening substances

Starch may be detected by means of iodine.

Gelatin may be detected by means of Stokes' reagent (prepared by dissolving mercury in twice its weight of nitric acid of sp. gr. 1·42 and diluting with water to 25 times its volume) in the following manner. Ten ml of the cream, 20 ml of water and 20 ml of the reagent are mixed together, shaken vigorously, allowed to stand for 5 minutes, and filtered; in the presence of much gelatin it will be impossible to obtain a clear filtrate, whilst the addition of an equal volume of a saturated aqueous picric acid solution to the filtrate will produce immediately a yellow precipitate.

Calcium saccharate (" *Viscogen* ") is best detected by determining the percentage of lime in the ash, which is normally 22·4 per cent (Baier and Neumann, 1908). Pyne (1930) has devised a method which will demonstrate the existence of thickening due to " Viscogen ". This is based on the decrease in viscosity caused by the addition of 1 ml of a saturated solution of neutral potassium oxalate to 40 ml of the thickened cream.

Glycerin—It is possible that this might be used as a thickening substance. According to Lerrigo (1928) its presence should be suspected if the total solids fume on being taken from the oven after 30 minutes' heating, if the total solids continually lose weight after several hours of drying, or if they assume an unusually brown appearance.

Sucrose can be detected by the methods previously described under Milk, p. 413.

Buttermilk and whey

These are analysed by the methods given for milk. If the Gerber process is used for the determination of fat in buttermilk, the butyrometer should be re-whirled in order to obtain the maximum fat reading.

ANALYSIS OF CONDENSED MILK

The Public Health (Condensed Milk) Regulations, 1923, require that all tins of condensed milk of less than 5 lb gross weight shall bear a label in a specified form indicating whether the contents are made from milk or skimmed milk, what quantity of fresh milk or skimmed milk the contents are equivalent to, and whether the product is sweetened or unsweetened. The analysis of condensed milk is usually carried out with a view to the verification of the above points, although it may also be necessary to examine the contents of the tin for injurious metals, etc. The determination of the equivalent of fresh milk depends entirely upon the determination of total milk-solids giving a result which corresponds exactly with the total solids of the original milk, due allowance being made for the degree of concentration; with sweetened products it also depends on the accurate determination of sucrose and its degradation products. These two determinations have been very thoroughly investigated by the Milk Products Sub-Committee of the Society of Public Analysts, and their recommendations have been published in the form of three reports (S.P.A. 1927, 1930, 1932). The methods therein described are reproduced in the following paragraphs and should be always employed when there is any dispute as to the equivalent of fresh milk in the contents of a tin.

Sampling

Before opening the tin, its gross weight should be determined to the nearest gram (after the contents have been transferred to a sample jar the tin can be washed out, dried, and its weight determined). The tin is then opened and its contents carefully mixed with a spoon in such a manner as to ensure that the top and bottom layers are thoroughly mixed; any separated crystals which may be felt should be ground up and carefully incorporated in the bulk. Care should be taken to avoid the formation of air bubbles. The S.P.A. recommends that weighed quantities of the mixed sample should be taken for each determination, in preference to making up a dilution and taking aliquot parts by volume, because of the difficulty of accurately measuring volumes of the diluted condensed milk.

Calculation of contents of tins

After the analysis is complete, the percentage of total milk-solids and the percentage of fat are converted into total grams of milk-solids and fat in the tins, respectively, by multiplying the percentage figures by the weight of the contents of the tin in grams over 100. The figures obtained should not be less than the figures given in Table 22.2, corresponding to the equivalent in pints indicated on the label.

The figures given for whole milk are based on 12·4 per cent of total solids, including 3·6 per cent of milk fat, and assuming a specific gravity of 1·032.

Table 22.2—Grams of total solids and fat in pints and fractions of a pint of milk

Pints		$\frac{1}{2}$	$\frac{5}{8}$	$\frac{3}{4}$	$\frac{7}{8}$	1	$1\frac{1}{8}$	$1\frac{1}{4}$	$1\frac{3}{8}$	$1\frac{1}{2}$	$1\frac{5}{8}$	$1\frac{3}{4}$	$1\frac{7}{8}$	2
Whole milk	Fat ..	10·5	13·1	15·8	18·4	21·1	23·7	26·3	29·0	31·6	34·3	36·9	39·6	42·2
	Total solids	36·3	45·4	54·5	63·5	72·6	81·7	90·8	99·9	109·0	118·1	127·2	136·2	145·2
Skimmed milk	Total solids	26·4	33·0	39·6	46·3	52·9	59·5	66·0	72·7	79·3	85·9	92·5	99·2	105·8

For skimmed milk, it was assumed that 9 per cent of solids-not-fat were present, that the milk fat content was negligible, and that the specific gravity was 1·035.

Determination of total solids—(1) S.P.A. method (1927, 1930, 1932)

Preparation of the support. Select for use sand which passes a 30-mesh and is retained by a 90-mesh sieve. Heat a convenient quantity of this sand with strong hydrochloric acid to remove oxide of iron, etc.; decant; repeat the digestion till the acid liquor is nearly colourless; wash, once with dilute hydrochloric acid, and then thoroughly with distilled water; dry, and ignite.

The sand thus prepared should be tested for suitability as follows. Dry a portion at 98° to 100° C and weigh; moisten with distilled water, and subsequently dry again at 98° to 100° C. There should be no difference between the two weights.

Dishes. These should be of metal (nickel is suitable), with readily removable but close-fitting lids; a suitable size is of diameter about 3 inches and depth about 1 inch.

PROCEDURE

(1) *Sweetened condensed milk.* Place about 25 g of the prepared sand and a short glass stirring-rod in the dish and dry to constant weight in an oven at 98° to 100° C, the lid being removed whilst drying and replaced before removing the dish from the oven. Allow the dish to remain for 45 minutes in the desiccator before weighing.

Tilt the sand to one side of the dish; place on the clear space about 1·5 g of the well-mixed sample and weigh rapidly. Add 5 ml of water to the milk and mix these; then mix the diluted milk thoroughly with the sand by means of the rod.

Place the dish on a rapidly boiling water-bath for 20 minutes, carefully stirring during the earlier period. Transfer the dish, with rod and cover, to a well-ventilated oven at 98° to 100° C., as recorded by a thermometer in the air immediately above the dish. After 1½ hours, cover the dish and place in the desiccator for 45 minutes; weigh; return the dish to the oven, and heat for one hour with lid removed; remove and weigh as before; repeat this process until the loss of weight between successive weighings does not exceed 0·0005 g.

(In a satisfactory determination it is generally found that the loss between the second and third weighings does not exceed 0·0005 g.)

(2) *Unsweetened condensed milk.* Weigh out 3 g of condensed milk and use 3 ml of water; otherwise proceed as in (1).

(2) **The A.O.A.C. (1950) method** for unsweetened evaporated milk directs that a dilution of 40 g of the mixed sample and 60 g of water be prepared, and 4 to 5 g of the mixture are dried at the temperature of boiling water in a weighed, flat-bottomed platinum dish, with or without the addition of sand.

For sweetened condensed milk, either a 1-in-5 dilution or separate portions of the mixed sample are dried at the temperature of boiling water on sand or asbestos fibre in a 5-cm flat-bottomed dish.

The dishes are not fitted with lids, but they are allowed to cool in a desiccator and then weighed quickly to avoid the absorption of water.

Fat—The Röse-Gottlieb process is the only completely satisfactory method for the determination of fat in either sweetened or unsweetened condensed milk. The modification of this method recommended by the S.P.A. is given on p. 339.

For routine determinations of fat the Gerber process may be used. Details of the procedure are given on p. 345.

Ash—Weigh about 5 g of the sample, dry, and ash at a low red heat exactly as for fresh milk.

Proteins—Weigh about 2 g of the sample on filter-paper, transfer to a Kjeldahl flask, and then proceed as for milk. Multiply the percentage of nitrogen found by 6·38 to give percentage of proteins.

Determination of sucrose

Next to the determination of total solids, this is the most important determination carried out on sweetened condensed milks. A criticism of the methods available is given in Report No. 2 of the S.P.A. (1930) Sub-Committee, and the method recommended is a polarimetric one, using zinc acetate–potassium ferrocyanide as the clarifier; this method is reproduced on p. 371.

A somewhat less accurate determination can be carried out by a modification of Vieth's method, described on p. 370. To 100 ml of a 20 per cent solution of the sample add 3 ml of the acid mercuric nitrate reagent, shake vigorously, and filter. Polarise in a 200 ml tube. Invert 50 ml of the clarified solution by immersing in a boiling water-bath for 12 minutes. Cool to 20° C, make to bulk if necessary, filter, and again polarise in a 200 ml tube. Calculate $[\alpha_D]$

before and after inversion, which, for a 20 per cent solution, a 200 ml tube and readings in angular degrees taken by sodium light, will be—

$$[\alpha]_D = \frac{\text{reading} \times 100}{2 \times 20}.$$

The difference of the $[\alpha]_D$ before and after inversion $\times \frac{100}{87}$ = percentage of sucrose.

This result is approximately corrected for the volume of protein by the volume of acid mercuric nitrate which was added, but it must also be corrected for the volume of fat, as described on p. 371.

The A.O.A.C. (1950) method for the determination of sucrose also employs acid mercuric nitrate as the clarifying agent, but differs from the Vieth process in that the solution is neutralised with 0·5 N sodium hydroxide before filtering; a portion is inverted by the addition of hydrochloric acid and standing at room temperature and corrections for both protein and fat are made; it is assumed that 1 g of protein occupies a space of 0·8 ml, and 1 g of fat, 1·075 ml.

Determination of original sucrose in sweetened condensed milk in which the sucrose has altered during storage

The products formed from the sucrose are dextrose, laevulose and laevan. If, however, the solution is inverted, the whole of the sucrose, dextrose, laevulose and laevan are found to be present solely as invert sugar, and in the same quantity as would have been given by the original sucrose had none undergone alteration. It therefore follows that after a solution of sweetened condensed milk has been inverted, the only sugars present are lactose and invert sugar. The amounts of these may be calculated from the results of two independent determinations, and the amount of invert sugar is then readily calculated to the amount of original sucrose. The two determinations recommended by the S.P.A. (1932) for the calculation of original sucrose are the measurement of the specific rotation of the inverted serum and the carrying out of a chloramine-T titration. Details of the processes and calculations are given on pp. 385.

Should it be considered necessary to determine the amount of laevulose, as distinct from laevan, present in the sample, Hinton and Macara's method may be used. Details of this process are given on p. 383.

In order to demonstrate the presence of reducing sugars formed from cane sugar, the S.P.A. modified Barfoed reagent, described on p. 380, should be used.

Lactose

This constituent is usually determined by the method of difference, because of the difficulty, previously mentioned, of deciding upon the exact amount of combined water accompanying the lactose in the total solids. Should a determination be necessary, it may conveniently be made at the same time as that of sucrose, using either of the polarimetric processes described for sucrose in sweetened condensed milk. The $[\alpha]_D$ due to sucrose, if any, is calculated by multiplying the percentage of sucrose by $\frac{66 \cdot 5}{100}$. The figure thus obtained is subtracted from the $[\alpha]_D$ before inversion of the sample, and the difference is multiplied by $\frac{100}{52 \cdot 5}$, giving the percentage of lactose in terms of hydrated lactose.

For other methods of determining lactose, sucrose and invert sugar in sweetened condensed milk, the report by G. W. Monier-Williams (1930) should be consulted.

Citric acid—This may be determined by the pentabromoacetone method as modified by Lampitt and Rooke. Details of this method are given on p. 388; a further modification of the process, especially suited to sweetened condensed milk, due to Arup, is also described.

Tin—The process described for tin in wrapped cheese on p. 517 may be used. Unsweetened condensed milk acts more readily on the interior of the tins than does the sweetened product.

THE ANALYSIS OF DRIED MILK

The Public Health (Dried Milk) Regulations, 1923, require that all tins or other packages of dried milk of less than 10 lb weight should bear a label declaring (*a*) whether the powder is full cream, three-quarter cream, etc., and (*b*) the equivalent number of pints of fresh milk to which the contents correspond. The regulations also apply to any powders which contain not less than 70 per cent of dried milk. The analyst, therefore, has to determine (i) whether the powder has been prepared from full-cream milk, (ii) whether the package contains sufficient milk powder to make the equivalent of the stated quantity of fresh milk, and (iii) whether, in the case of mixtures, there is sufficient milk powder present to bring the commodity within the scope of the Dried Milk Regulations.

Sampling

From the above the importance of determining the weight of the contents of all packages of dried milk before analysis will be appreciated. After weighing the contents they should be rapidly and thoroughly mixed, and the portion for analysis immediately transferred to an airtight sample jar. This is important, as the moisture content of dried milk increases rapidly on exposure to the atmosphere.

Calculation of contents of packages

After the analysis has been completed, i.e. after the moisture and substances foreign to milk have been deducted, it is possible to convert the percentage of milk fat and milk solids into equivalent pints of liquid milk. This will be facilitated by the use of Table 22.3, which gives the grams of fat and total solids which should be yielded by 1 pint of milk of the indicated cream content.

Determination of moisture

This is the most important determination to be made in the case of pure dried milks, when it is necessary to ascertain the equivalent in pints of fresh milk of the contents. The moisture figure subtracted from 100 will give the percentage of total milk-solids, and in order that this should be directly comparable with the total solids of fresh milk it is important that the determination

Table 22.3—Grams of fat and total solids in one pint of milk

	Full cream	Three-quarter cream	Half cream	Quarter cream	Skimmed
Total solids (g)	72·69	68·08	63·37	58·16	52·91 (s.n.f.)
Fat (g)	21·11	15·84	10·55	5·27	—

This table is based on the following figures for the original milk—

	Full cream	Three-quarter cream	Half cream	Quarter cream	Skimmed
Total solids per cent ..	12·4	11·6	10·8	9·9	9·0 (s.n.f.)
Fat per cent ..	3·6	2·7	1·8	0·9	—
Specific gravity	1·032	1·033	1·034	1·034	1·035

should be carried out in such a manner as to give solids which are as nearly as possible in the same condition, as regards combined moisture, etc., as total solids determined on fresh milk. The following method, due to the Milk Products Sub-Committee of the S.P.A. (1936a) conforms to the above requirement.

Dishes. These should be of metal (nickel is suitable) with close-fitting but easily removable lids; diameter 2 in. approximately and depth 1 in. approximately.

PROCEDURE. Uncover the dish, and place dish and lid in the oven at 102° to 103° C for 1 hour. Place the lid on the dish, remove from the oven, cool in a desiccator for 30 minutes and weigh.

Transfer approximately 1 g of the well-mixed sample to the dish, cover with the lid, and weigh accurately and rapidly.

Remove the lid, place both dish and lid in the oven and maintain at 102° to 103° C for 2 hours.[1] Replace the lid, remove from the oven, and allow to cool in the desiccator for 30 minutes; weigh.

In the same manner heat again in the oven for 1 hour and repeat this process until the loss of weight between successive weighings does not exceed 0·0005 g (generally, drying is complete at the end of the first two hours).

The maximum loss of weight found is the weight of water in the quantity of sample taken, and the percentage of total solids is 100 minus the percentage of water thus found; where the sample consists of a dried milk to which no other substance has been added, these solids will be the total milk-solids including fat, mentioned in the Dried Milk Regulations.

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[1] With samples of high moisture content, the first heating may be advisedly extended to 3 hours.

Particular attention should be given to checking the temperature of the interior of the oven; the dishes should not be placed near the sides of the oven and they should not rest directly on metal shelves.

The A.O.A.C. (1950) also suggests a direct drying method, but the apparatus required is more complicated in that the dish and contents are dried in a vacuum oven at 100° C under a pressure not exceeding 100 mm of mercury.

Kumetat and Demmler (1950) have found that the calcium carbide and Fischer methods can be successfully used for butter and cheese, but the Fischer method is unsuitable for milk powder.

Ash and mineral constituents are determined as for milk by carefully igniting 5 g of the sample at a low temperature.

Proteins. One g is used for the nitrogen determination by the Kjeldahl process. For calculating proteins from nitrogen the usual milk protein factor of 6·38 should be used.

Determination of fat

Fat cannot be determined by direct extraction of the powder (Jephcott 1923). The Werner-Schmid or the Röse-Gottlieb process will give satisfactory results. The best method is, however, the process recommended by the Milk Products Sub-Committee of the S.P.A. (1936a) which incorporates the advantages of both processes and is given in detail below.

REAGENTS. Hydrochloric acid—Sp. gr. 1·16.
Concentrated ammonia solution—Nominal 0·880.
Alcohol or industrial methylated spirit—About 95 per cent by volume.
Ether (methylated)—Sp. gr. 0·720.
Petroleum spirit—Boiling between 40° and 60° C.
These reagents should leave no appreciable residue on evaporation.

PROCEDURE. Transfer to a hard glass boiling-tube (8 × 1 in.) (*Note* 1, *below*) approximately 1 g, accurately weighed, of the well-mixed sample; add 8 ml of water and 2 drops of the ammonia solution. Gently boil the mixture until all lumps are disintegrated. Add 10 ml of the hydrochloric acid, and heat in a Bunsen flame with gentle agitation; after the liquid begins to boil, continue gentle boiling for 3 minutes. Cool; add 10 ml of the alcohol, and mix well. Add 25 ml of ether, close the tube with the water-moistened stopper (*Note* 2) shake well for 15 sec. Cool (*Note* 3), remove the stopper; wash the stopper and the neck of the tube with petroleum spirit, and add, including the amount in the washings, 25 ml of petroleum spirit. Replace the re-moistened stopper, shake vigorously for 30 sec., and either allow the tube to stand or whirl in a centrifuge until the two layers of liquid are completely separated.

Transfer the ethereal layer as completely as possible to a suitable flask by means of a siphon or wash-bottle fitting. Wash the tip of the siphon-tube (into the flask) with ether; disconnect the siphon-fitting and wash down the inside of the extraction-tube with 5 ml of ether; without further shaking, siphon off this ether, and wash the tip of the fitting as before (Fig. 6).

Add 15 ml of ether to the extraction-tube, using this ether to wash the cork and inner limb of the siphon-fitting before its removal. Replace the freshly-moistened stopper; shake for 15 sec.; add 15 ml of petroleum spirit and shake for 15 sec., taking the same precautions as to washing the neck and stopper as before. When the ethereal layer has separated, transfer it to the flask as before.

Repeat the extraction with 15 ml of ether and 15 ml of petroleum spirit and the transference of the ethereal layer to the flask as in the last paragraph, and wash the tip of the siphon-tube.

Cautiously distil the solvents from the flask, and then dry the residual fat at 102° to 103° C for 1 hour, removing all solvent vapour from the flask at the early stages of the drying by blowing air gently into the flask. Cool and weigh. Repeat the heating until there is no loss in weight.

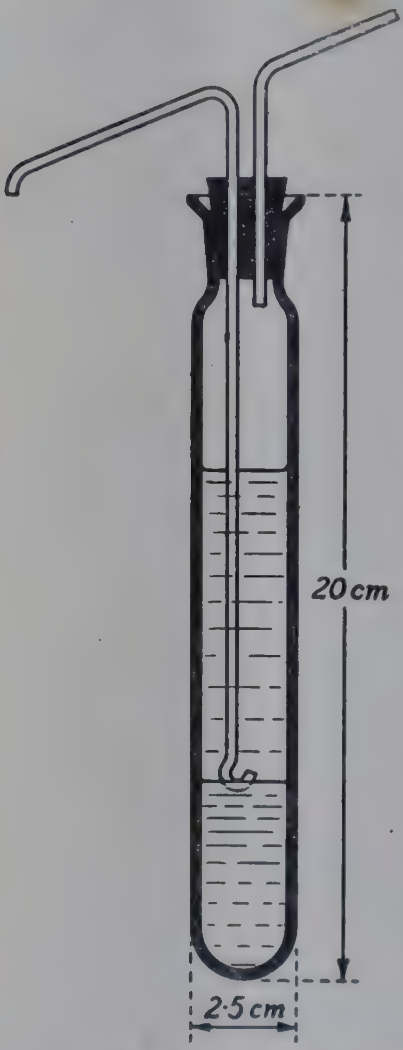

Fig. 6—Apparatus for determination of fat in dried milk by the S.P.A. method

(Reproduced by permission of the Society)

Completely extract the fat from the flask by repeated washings with petroleum spirit, allowing any sediment to settle before each decantation, and washing off any fat which may have crept over the edges of the flask during the removal of the fatty solutions (*Note* 4). Dry the flask at 102° to 103° C, with removal of solvent vapour, cooling and weighing as before.

The difference in weight before and after the petroleum spirit extraction is the weight of fat contained in the quantity of dried milk taken for analysis, uncorrected for the blank.

Make a blank determination, using the specified quantities of reagents and distilled water, and deduct the weight found, if any, from the weight of fat obtained.

Notes. The success of the method depends upon close attention to detail.

(1) The use of a boiling-tube, into which the dried milk is introduced and in which it is dissolved, avoids the possibility of loss during transference, such as may occur when the milk is digested in one vessel and transferred to another vessel for extraction. A narrow neck to the boiling-tube permits the use of a small stopper for closing the tube and for the wash-bottle fitting. A funnelled mouth facilitates the introduction of the sample into the tube, and has the additional advantage that any trace of solution which may pass the stopper, when it is released, is retained in the funnel and can be readily washed back into the tube.

(2) Sound, well-fitting corks only should be used, if the glass-stoppered tube is not available. Rubber bungs are not suitable. The stopper or cork should be moistened with water before insertion for each extraction.

(3) Before each operation of removal of stopper or cork, a slightly reduced pressure in the tube should be induced by cooling, in order to avoid spurting of the solvent.

(4) The non-fatty residue carried over with the ethereal solutions should be very small. Difficulty lies in preventing the flotation of this residue in the petroleum spirit in the final operation. The addition of a few drops of water

to the ethereal liquid before distilling off the solvents is of service in concentrating this non-fatty substance, if any, into a small compass and causing it to adhere to the flask.

The acidity is usually determined by treating from 1 to 2 g with about 10 ml of water at 50° C and titrating with 0·1 N strontia or caustic soda solution to phenolphthalein.

Determination of lactose

When determined by the usual methods, lactose gives a figure which, when added to the other constituents expressed as percentages, does not add up to 100. This, according to Richmond (1920), is due to the fact that it is difficult to assign a correct factor for the lactose, as both the hydrated and anhydrous sugar may be present, although usually (but not always) the bulk of the milk sugar is present as hydrated sugar.

For ordinary purposes it will, however, be sufficiently accurate to determine lactose by dissolving 20 g of the sample in about 150 ml of water at 60° C in a 200-ml capacity graduated flask and then proceeding by the S.P.A. polarimetric method for sucrose in sweetened condensed milk, which is given on p. 371. After adding ammonia, acetic acid and the precipitating agents, the contents of the flask are, of course, made up to 200 ml before filtering.

Alternatively, the polarimetric method of Vieth may be used (see p. 370), taking 10 g of dried milk made up to 100 ml after dissolving in warm water and then adding 3 ml of acid mercuric nitrate.

Copper reduction methods can, of course, also be used, and the following modification of the Brown, Morris and Miller method (see p. 378) has been found convenient by Jephcott (1923).

A solution of the powder, 3 g in 250 ml, is made up in the ordinary way with hot water, heated to boiling, and cooled to atmospheric temperature. Fehling's copper solution is used as precipitant, the quantity taken being just slightly in excess of that needed to produce complete precipitation. The solution is then made up to the required volume, and shaken very vigorously for about 30 sec. After filtration through a pleated filter-paper, 50 ml of the solution are reduced with 50 ml of Fehling's solution for 30 min. at 80° C in a 240-ml " Kavalier-glass " beaker, the beaker being immersed in a water-bath so that the level of the water outside is the same as that of the solution inside; the beaker is covered with a clock-glass of appropriate size. After the half-hour, the beaker is removed from the bath, the clock-glass rinsed with hot water, and the whole solution filtered through a porcelain Gooch crucible. The precipitate is collected in the usual manner and finally washed with a little 95 per cent alcohol. After 10 minutes' drying in the oven at 102° C the crucible is ignited inside a larger silica crucible or in a muffle, and the weight of cupric oxide determined after the crucible has been cooled in a desiccator. Then

$$\text{Percentage of lactose} = \text{CuO (in grams)} \times 106{\cdot}5$$

This result needs to be corrected for the volume of precipitated fat and protein.

Sucrose. This can be determined conveniently, if present, at the same time as lactose by following the S.P.A. polarimetric method (see Lactose, above, and p. 371).

Determination of solubility

Lampitt and Hughes (1924) determined the insoluble matter by the following method, and returned the results as percentage solubility of the solids-not-fat (thereby allowing for the varying moisture and fat contents of different samples).

Five grams of full-cream powder are treated with 38 ml of distilled water at 20° C in a flask of 250-ml capacity by shaking steadily for 3 minutes (5 g of skim-milk powder are treated with 45 ml of water). The whole of the material is transferred to a tared centrifuge tube and whirled at 1,500 revolutions per minute for 3 minutes. The layer of cream is discarded, and, without disturbing the deposit, 5 ml of the liquid are withdrawn and tared in a milk dish and the solids (A) determined. As much as possible of the remaining liquid, with any cream adhering to the side of the tube, is carefully discarded. The deposit and small amount of residual liquid are weighed in the tube, thus obtaining the weight of deposit and liquid. This is washed with distilled water into another milk dish, and the solids (B) again determined. From the amount of moisture which is lost in this operation the amount of soluble matter present in conjunction with the insoluble matter (in B) can be calculated from the figures given by the first determination of solids (A) on the liquid; this, subtracted from the solids (B), will give the amount of insoluble matter obtained in grams from 5 grams of the powder.

Parsons (1949) has published the methods which he uses for the routine testing of the solubility and other properties of milk powders. His technique is as follows—

(1) *Reconstitution*

Proportions	Separated powder 30 g to 300 ml of water. Full cream powder 37·5 g to 300 ml of water.
Temperature of water ..	For spray powder: 20° C For roller powder: 85° C
Method of mixing	Place the water hot or cold in a mixing vessel, add the powder and mix with an electric mixer for 30 sec.

(2) *Titratable acidity*

To 10 ml of reconstituted milk add 1 ml of 0·5 per cent solution of phenolphthalein in 50 per cent alcohol. As a colour standard, to a second 10 ml of milk add 1 ml of bench solution of rosaniline acetate (see below). Titrate the 10 ml to which phenolphthalein has been added with N/9 NaOH solution. If titration is *T* ml then T/10 = titratable acidity expressed as grams of lactic acid per 100 ml of reconstituted milk.

Rosaniline acetate solution

(*a*) Stock solution. Dissolve 0·12 g of rosaniline acetate in approximately 50 ml of ethyl alcohol (95/96 per cent v/v) containing 0·5 ml of glacial acetic acid. Make up to 100 ml with industrial alcohol.

(*b*) Bench solution. Dilute 1 ml of the stock solution to 500 ml with a mixture of ethyl alcohol 95/96 per cent and water in equal proportions by volume.

Note. Solutions (*a*) and (*b*) shall be stored in the dark in bottles securely stoppered with rubber bungs.

(3) *Burnt particles, foreign matter and quality*

Place 250 ml of the reconstituted milk in a tall beaker and allow to stand overnight. Examine the bottom of beaker for burnt particles, dirt, etc. Judge comparative solubility and quality of milk from quantity, appearance, and density of deposit, and from appearance (colour, translucency, etc.) of the liquid above the deposit.

(4) *Moisture*

Dry 5 g in oven at 100° C for 5 hr. The oven is fitted with a fan to equalise the temperature.

(5) *Butter fat*

For routine purposes determine by Gerber process as described in B.S. No. 696. In cases of dispute use the S.P.A. method (*Analyst*, 1936, p. 110).

(6) *Determination of solubility of roller-dried milks*

(*a*) *Reconstitution.* Weigh out approximately 4 g of powder into a 50 ml Pyrex boiling tube. Add 32 ml of water at 50° C. Cork tube and shake for 10 sec. Place in a water bath at 50° C for 5 min. Shake for 1 min. In shaking make 4–6 double excursions of about 12 in. per sec.

(*b*) *Removal of cream* (not applicable to separated powders). Completely fill a centrifuge tube with the hot reconstituted milk and centrifuge for 10 min. in the bowl centrifuge. Cool in refrigerator until the fat is solid but do not freeze the milk. Remove the fat layer with as little milk as possible by running a needle around the edge of fat and then removing it with a spoon-shaped spatula. Warm the milk to 20° C. Break up deposit with a rod or wire, and cork tube and shake well until the liquid appears homogeneous.

(*c*) *Determination of total reconstitutable solids.* Transfer about 2 ml of the milk after removal of the fat and redispersal of the precipitate to a tared covered metal dish and weigh. Let weight of liquid equal L_1. Place covered dish aside for drying with second total solids. Centrifuge tube again for 10 min. Pipette about 2 ml of upper layer into a second covered dish without disturbing sediment and weigh. Let weight of liquid be L_2.

(*d*) *Drying.* Uncover both dishes and place side by side on steam bath until apparently dry. Place in oven at 100° C for $1\frac{1}{2}$ hr. Cover dishes, cool and weigh. Let weight of solids corresponding to L_1 and L_2 be S_1 and S_2.

$$\text{Then solubility} = \frac{\dfrac{S_2}{L_2}}{\dfrac{S_1}{L_1}} \times 100 \text{ per cent}$$

$$= \frac{S_2 \times L_1 \times 100}{S_1 \times L_2}$$

Notes. The centrifuge tubes used have a total capacity of approximately 25 ml. The centrifuge is operated at 2,000 r.p.m. and as the radius to the bottom of the rotating tube is about 17 cm the precipitating force used is approximately 769 g.

(7) *Solubility index* (spray powder only)

Measure 20 ml of the milk reconstituted as described under (1) into a graduated centrifuge tube of about 25 ml capacity (see above). Spin for 10 min. Without disturbing the sediment remove milk within 5 mm of top of deposit by means of a blow-off apparatus. Refill with water to the 20 ml mark, stir up sediment, shake well and again spin for 10 min. Read off volume of deposit and record as " Solubility Index ".

Some laboratories, following the lead of the American Dry Milk Institute, carry out a Solubility Index test on 50 ml of milk.

(8) *Ash and alkalinity of ash*

(*a*) Ash. Weigh 2 g of the powder into a silica or platinum dish of about 50 ml capacity and heat gently over a flame or at the mouth of the muffle until smoke is no longer given off. Place dish within the muffle and continue heating at 550° C for 1 hr. Cool in a desiccator and weigh.

(*b*) Alkalinity of ash. Ash 2 g of powder as described above. Add 10 ml of 0·2 N HCl from a pipette. Stir with a flat-ended glass rod and break up any particles. Warm carefully with stirring until all ash is dissolved. Cool and add 2 ml of neutral 40 per cent $CaCl_2$ solution and 1 ml of 0·5 per cent phenolphthalein solution in alcohol. Titrate with 0·2 N NaOH until a faint pink coloration remains just visible for 30 sec. on the addition of one drop of soda solution. Express the result as ml of 0·2 N HCl per 100 g of powder as received, and also as ml of 0·2 N HCl per g of ash.

Waite and White (1949), have shown that as milk powders become more insoluble, increasing quantities of fat are taken into the sediment and increasing quantities of protein into the fat. A washed sediment consists mainly of denatured calcium caseinate associated with tricalcium phosphate. These authors conclude that most published methods give reasonably accurate results with powders of high solubility, but none gives accurate results with roller powders or with spray powders of low solubility. The following comparative results illustrate this point.

Table 22.4—Comparative solubilities of milk powders

(The first value is for 20°, the second for 50° C)

Method	Roller A		Roller B		Kestner		Klim		Krause		Milkal	
	Solubility per cent											
Howat *et al.*	76	91	96	98	93	100	97	100	100	100	100	100
Lampitt and Hughes	75	80	95	100	93	100	97	100	100	100	100	100
Parsons	70	81	96	99	91	99	94	98	98	99	98	99
	Sediment volume per cent											
Amer. Dry Milk Inst.	10	11·5*	4*	0·4	0·6	<0·1	0·3	<0·1	0·1	<0·1	0·1	<0·1
Howat *et al.*	1·6	2·4*	3*	0·1	0·5	<0·1	<0·1	0·5	<0·1	<0·05	<0·1	<0·1

* Indicates that demarcation between layers was indistinct.

Strobel and Babcock (1951) have described a filtration method for the determination of sediment in roller skim milk. Their method is as follows.

Mix 25 g of sample with 100 ml of pepsin-hydrochloric acid solution[1]; place in 45° C water bath and hold 20 min.; add approximately 0·5 ml of caprylic alcohol or diglycol laurate (defoaming agents); bring to the boil within 5 to 8 min. and filter immediately through a 1·25-in. lintine disc (waffle side up).[2] Rinse sample container with boiling, or near boiling, water and pass rinse through the filter disc. The temperature of the sample solution should not be allowed to drop prior to boiling or prior to filtering. In the absence of a standard more representative of the material in dried milks, the quantity of sediment, including scorched particles, in the sample is determined by comparing the disc with the USDA Sediment Standards for Milk and Milk Products.[3]

The authors claim that this method gives results comparable with the normal disc method and that the new method is very satisfactory for measuring the burnt particles in this product.

THE ANALYSIS OF MALTED MILK POWDER

This product is prepared from whole milk and the liquid separated from a mash of barley malt and wheat flour, by evaporation under carefully controlled conditions. It should not contain any unconverted starch, cane sugar; skimmed milk, etc., and the diastase of the malt should be active.

Sampling. Like dried-milk powder, this product is hygroscopic and should be sampled quickly and kept in an airtight jar from which the portions for analysis are weighed rapidly.

The methods of analysis for moisture, ash, protein and fat are the same as those for dried milk. Should the sample contain any unconverted starch, the most convenient method for fat is that of the S.P.A., described under " Dried milk ".

Sucrose, if present, can be determined by inversion and double polarisation as described in the S.P.A. method for sucrose in condensed milk.

Determination of soluble extract

To determine total soluble extract, including lactose, malt sugars, soluble proteins and cane sugar, if present, 5 g of the sample should be placed in a 100-ml graduated flask, about 80 ml of 50 per cent by volume alcohol added, and the mixture shaken for 1 hour, made to bulk, and filtered. Total solids are determined on 20 ml (1 g sample) of the filtrate by drying in a tared milk dish containing ignited sand and a glass rod. Should it be necessary to differentiate lactose from other reducing sugars, the fact that lactose is not fermented by washed brewers' yeast, while dextrose and maltose are, may be used for this purpose. After fermentation is complete, the lactose remaining in the liquid may be estimated by its reducing action on Fehling's solution in the usual way.

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[1] Ten g of pepsin (powder (1 : 3,000) dissolved in approximately 500 ml of distilled water; 30 ml of C.P. HCl, 37 per cent, specific gravity 1·1878 added and solution made up to 1,000 ml with distilled water. Solution filtered. The pH of the solvent solution ranged from 0·5 to 0·7 during the experiment. Sample plus solvent pH ranged from 1·6 to 2·3.

[2] Aspirator was used in this experiment.

[3] The USDA Sediment Standards for Milk and Milk Products are made up of discs representing 0 mg; 0·025 mg; 0·050 mg; 0·075 mg; 0·10 mg; 0·20 mg; 0·30 mg; 0·50 mg; 1·00 mg; and 2·50 mg.

Determination of insoluble matter

An approximate figure for unconverted starch, etc., can be obtained by treating 1 g in a centrifuge tube with 20 ml of 50 per cent alcohol for 1 hour, centrifuging, and treating the residue with two further amounts of 20 ml of 50 per cent alcohol. The deposit is washed with alcohol and then with ether to remove fat and is finally dried in the oven and weighed. A Kjeldahl nitrogen determination should be carried out on the residue, and the equivalent amount of protein deducted, the remainder being returned as insoluble carbohydrate, etc. The amount of insoluble protein deducted from the total protein will give some idea of the amount of soluble protein present in the soluble extract. The factor for converting nitrogen to protein is selected after the sample has been examined microscopically and after an approximate idea of its composition has been obtained from other analytical figures.

More accurate determinations for starch are the iodine method and the taka-diastase method. A description of both these processes is given in a paper by Chinoy (1938).

Microscopical appearance

In order to distinguish between genuine malted milk and mechanical mixtures of milk powder, dried malt extract, etc., a small portion of the sample should be mounted in mineral oil on a microscope slide and the appearance of the particles noted. With genuine malted milk, these are angular and homogeneous in appearance and quite unlike mixtures containing dried-milk powder. In carrying out this examination it is advisable to compare the sample with other specimens of known origin and composition. Reference should also be made to the photomicrographs in *A.O.A.C.* (1950).

Determination of diastase

The presence of active diastase in the malt can be demonstrated by preparing without heat a 5 to 10 per cent filtered solution of the sample and adding a portion of this to varying amounts of a 2 per cent solution of pure soluble starch (prepared by dissolving soluble starch in boiling water and cooling to 20° C before use). The contents of the tubes are heated for 1 hour at 21° C and then tested against a blank with iodine for the presence of unconverted starch. Should any of the tubes fail to give a reaction for starch, the diastase must be active. If it is desired to obtain a quantitative value for the diastatic activity (or Lintner value), the method given in *Methods of Analysis for all English Malt Extracts*[1] should be followed.

The following example will indicate how the approximate composition of a so-called malted milk powder was arrived at from data obtained by the above methods.

Microscopically, the powder was found to contain unconverted wheat flour, and it had the appearance of a mechanical mixture containing dried-milk powder. The powder gave the following figures upon analysis: fat, 1·4 per cent; total proteins, 26·8 per cent; ash, 4·4 per cent; moisture, 5·6 per cent;

[1] H.M. Stationery Office, S. R. & O., 1933, No. 540.

insoluble in 50 per cent alcohol, 35·9 per cent of which 14·3 per cent was protein, therefore 21·6 per cent starch; soluble extract, 53 per cent, of which 26·8 — 14·3 = 12·5 was protein, therefore 40·5 per cent represents soluble carbohydrates (this figure agrees with the figure of 40·2 per cent obtained by subtracting all the other constituents of the sample, including starch, from 100). The high protein figure indicates a considerable proportion of dried-milk powder, which, in view of the low fat content, is probably skimmed-milk powder. Wheat flour contains approximately 73 per cent starch and 12 per cent protein. Therefore 21·6 per cent of starch in the sample corresponds to approximately 25 per cent of dried wheat flour, and 3·6 per cent of the protein of the sample is derived from this source. There remains, therefore, 23·2 per cent of proteins and 40·5 per cent of soluble carbohydrates (sugars) to be reported in terms of dried malt extract and skimmed-milk powder. Assuming that average figures for dried malt extract are proteins 6·2 per cent and soluble carbohydrates 93 per cent, and for skimmed-milk powder proteins 36 per cent and lactose 50 per cent, the approximate composition of the powder was 60 per cent skimmed-milk powder, 15 per cent dried malt extract and 25 per cent wheat flour. Assuming that no additional mineral matter has been added, a rough check can be obtained from the ash, which for a skimmed-milk powder is approximately 7·0 per cent, for wheat flour 0·4 per cent and for dried malt extract 1·8 per cent.

Infants' foods, malt beverages, etc.

The analysis of these follows the lines adopted for dried-milk powder and malted-milk powder. Many of these beverages contain cocoa powder, which can be detected from its microscopical appearance. The amount of cocoa may be calculated from a determination of total cocoa alkaloids by the method of Moir and Hinks (1935).

The determination of woody fibre is often helpful in arriving at the approximate content of cocoa and other substances which contain husk. Prepared cocoa powder contains approximately 5·5 to 6·5 per cent of fibre, calculated on the dry, fat-free sample.

METHOD. Two to three grams of the sample are defatted by ether extraction and transferred to a 600-ml beaker. Two hundred ml of 1·25 per cent hot sulphuric acid are added and the mixture boiled gently for exactly 30 minutes. It is then filtered, with the aid of gentle suction, on a Buchner funnel, lined with a circular piece of cotton cloth on which rests a filter-paper. After washing the residue with hot water, it is washed back into the beaker with 200 ml of 1·25 per cent hot caustic soda solution, which for convenience is contained in a wash-bottle. After boiling gently for a further 30 minutes, the solution is filtered through a No. 4 Whatman filter-paper, washed thoroughly with hot water, with a little 1 per cent hydrochloric acid, then with more hot water, and finally with alcohol, followed by ether. When partially dry, the residue should be transferred to a platinum or porcelain dish and dried at 100° C until constant in weight. It is then ashed, and the weight of the dish and ash deducted from the first weight, thereby obtaining the weight of the fibre. In order to obtain consistent results, it is important to adhere strictly to the strength of acid and alkali indicated and to boil exactly for the specified time.

THE ANALYSIS OF COMMERCIAL LACTOSE

Lactose is manufactured in large quantities from the whey from cheese-making and the liquid from the preparation of casein. The liquid is acidified, if not already sufficiently acid, heated to boiling and filtered. The clear liquid is concentrated in vacuum pans at about 60° until the solid content is about 60 per cent, when crystallisation is allowed to take place. The first crop of crystals is drained in a hydro-extractor and refined by re-crystallisation in the presence of a little bone-black. Commercial lactose may contain an amount of the pure substance varying from 78 per cent to 99·9 per cent; purity, of course, depending upon the mode of preparation and the method of refinement. The tests usually applied are determinations of moisture, ash, rotatory power, acidity, fat, and nitrogen. Of these, moisture is by far the most difficult. Lactose contains one molecule of water of crystallisation, which is driven off slowly at 100° but rapidly at a temperature of 130° C. Pure hydrated lactose contains exactly 5 per cent of water of crystallisation, but it is not safe to determine hygroscopic water by subtracting 5 from the total percentage of water, since any given sample may contain a mixture of hydrated and anhydrous lactose. The best method of carrying out the determination of hygroscopic moisture is probably by exposing the final ground sample in a vacuum desiccator for twenty-four hours; a similar figure can be obtained by heating the sample for about 2 hours in a well-ventilated oven at 90°. The following may be taken as the usual figures likely to be obtained from commercial samples—

Table 22.5—Composition of commercial lactose

Determination	Type of sample	
	Good	Average
Moisture	0·1 per cent	Not above 2 per cent
Ash	0·1 ,,	Not above 2·5 ,,
Nitrogen	0·01 ,,	Not above 0·06 ,,
Acidity	Mere traces	Not above 1·5 ml 0·1 N per 5 g

Starch should be absent, and the sample should give a polarisation reading in conformity with the amounts of impurity found.

Determination of total moisture

This may be carried out as directed above by heating at 130° C; or, alternatively, total moisture (free and combined) can be determined by Tate and Warren's (1936) modification of the Dean and Stark process, as described on p. 483. A solvent of somewhat higher boiling-point than commercial heptane is, however, required, and Tate and Warren find that dehydration is complete after three hours' boiling with light petroleum (B.P. 120° C).

Acidity. Dissolve 5 g in about 50 ml of distilled water and titrate with 0·1 N alkali to phenolphthalein. Each ml of 0·1 N alkali equals 0·009 g lactic acid. The British Pharmacopoeia standard requires that not more than 0·5 ml of 0·1 N NaOH should be used in the above test.

Another test for acidity can be made by dissolving 10 g of the sample in 100 ml of milk; this is brought to the boil; the milk should not be curdled.

Ash. Ignite 5 g in a platinum or porcelain dish carefully at a dull red heat until a white ash is obtained, and weigh. The British Pharmacopoeia maximum limit for the ash is 0·1 per cent. Should the complete analysis of the ash be required, this can be carried out exactly as described for the examination of milk ash on pp. 322 to 325.

Fat. This can be determined by the Röse-Gottlieb process as described for milk or dried milk.

Protein. Add a little acid mercuric nitrate solution to a 10 per cent solution of the sample in water; the solution should not show more than the faintest turbidity.

Other impurities. A solution of 3 g in 10 ml of water prepared by raising the mixture to boiling should be odourless, clear and colourless, or at the most faintly yellow.

Determination of lactose

Prepare a 10 per cent solution of the sample by boiling 10 g with about 80 ml of water for a few minutes in a 100-ml capacity flask, cooling to 20° C, clarifying, if necessary, with 3 ml of acid mercuric nitrate (see Protein, above), making up to 100 ml and filtering. Polarise this solution in a 200 mm tube.

Invert 50 ml of the solution by heating in a boiling water-bath for 12 minutes, cool, and again polarise in a 200 mm tube.

With pure lactose, the two readings should be practically identical. If any sucrose is present, however, the second reading will be lower than the first, and the amount of sucrose can be calculated as described under the Vieth method for sugar in milk, given on p. 370, remembering, of course, that in the present case we are dealing with a 10 per cent solution of the sample.

The British Pharmacopoeia requires the specific rotation at 20° C, determined as above, to be between $+ 52°$ and $+ 52·6°$.

Determination of the birotation ratio. Richmond describes the following method of carrying out this test. Add 6 or 7 g of the finely-powdered sugar to about 50 ml of distilled water; stir vigorously with a thermometer for ten seconds and allow the solution to settle for twenty seconds; read the fall in temperature on dissolution and filter the solution rapidly. When sufficient clear filtrate is obtained, fill a 200 mm polariscope tube, and polarise as soon as possible. Take polarimetric readings every minute till the specific rotatory power begins to diminish. If the temperature at which the solution is polarised is kept at 15° C, or below, there is no difficulty in obtaining several readings which are nearly constant, and the mean of these is taken as the *initial* rotation. Allow the tube to stand for twenty-four hours, and polarise again at the same temperature; this is the *normal* rotation. The initial rotation divided by the normal rotation will give the " birotation ratio ". The amount of milk-sugar in 100 ml of this solution is estimated either by drying 5 ml at 100° C, when a residue of anhydrous sugar will be left, or by deducing it from the normal rotation. This is done by dividing the reading in angular degrees by 1·106. The two figures should agree closely.

The British Pharmacopoeia[1] describes the following test for the detection of more soluble sugars. Shake 5 g of the sample with 20 ml of 90 per cent alcohol for 10 minutes, and filter. Evaporate 10 ml of the filtrate to dryness and weigh. The residue should not be more than 0·005 g.

Wagenaar (1934) suggested the following test for the detection of small amounts of sucrose in lactose, which depends on the fact that a solution of α-naphthol in glycerin gives, in the presence of sulphuric acid, a blue-violet condensation colour in the presence of a ketose group, or an orange condensation colour in the presence of an aldose group. Mix, by means of a platinum wire, a suspension of 5 mg of the sample in a drop of reagent with a drop of concentrated sulphuric acid on a microscope slide; a blue-violet colour after 10 minutes, particularly noticeable at the edge of the drop, indicates the presence of a ketose compound. If the test as carried out above appears negative, it is advisable to hold the slide over a boiling water-bath for about 30 sec, and then compare the resulting colour with that obtained by treating pure lactose under similar conditions.

The reaction is specific for laevulose or sucrose, and will detect 1 per cent or more of the latter. It may be made more sensitive by applying it to the residue left after evaporating the 90 per cent alcohol extract (above) of the sample which dissolves sucrose and other sugars in preference to lactose.

Detection of adulteration

Milk-sugars which are adulterated with other substances will show marked divergence from the figures given for pure commercial lactose on p. 474. Cane sugar can be detected by treating a solution with a little washed brewers' yeast and keeping at 35° C for five hours; milk-sugar shows no change in specific rotatory power, while the presence of even 1 per cent of sucrose will produce a marked alteration.

Dextrose is detected by an increase in the birotation ratio, by the solubility, and by a decrease in the fall of temperature.

Maltose and dextrin (present in commercial starch-sugar) are detected by a lowering of the birotation ratio, a great increase in the apparent percentage of milk-sugar, and in the solubility.

Mineral adulterants will be easily detected by the high percentage of ash.

Limit tests for copper, lead and arsenic are given in the B.P.

THE ANALYSIS OF COMMERCIAL CASEIN[2]

Casein is now used on the commercial scale in the manufacture of a large number of products such as plastics, glues, paper, paint, and pharmaceutical preparations; it is also made use of in the manufacture of textiles, leather and cosmetics. The industrial applications of casein are outside the scope of this work, but it seems desirable to give a short account of its preparation and xamination.

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[1] B. P. Addendum.

[2] For a complete discussion of the subject, see *Casein and its Industrial Applications* by E. Sutermeister and F. L. Browne, London: Chapman & Hall, 1939.

For the preparation of a good-class casein it is necessary to exercise every care so that a product of good quality may be obtained. Separated milk is used, the fat content of which has been reduced to the minimum quantity possible; oam should not be present. On the commercial scale, casein is precipitated by the addition of hydrochloric or sulphuric acids (the former when lactose is also to be recovered), by controlled souring, or by the action of rennet. The method used depends to some extent on the purpose for which the casein is required. Trimble and Bell (1933) state that the pH value should be about 4·1 in order that the curd produced shall be in the optimum condition for washing, draining, drying and grinding; the temperature should vary between 95° and 120° F, according to the method of precipitation adopted. After precipitation the curd is washed, drained (with or without pressing), ground, dried at as low a temperature as possible, and finally ground to the required degree of fineness.

Rennet casein, from its method of preparation, contains a higher proportion of ash than those obtained by precipitation with acid. The methods of analysis of casein include determinations of moisture, ash, lactose, acidity, nitrogen, and the observation of its physical condition, together with its solubility and the viscosity of its solutions.

Determination of ash

The mineral residue left on igniting casein may consist of inorganic materials not removed by washing, calcium oxide and calcium phosphate from the casein, and residue produced from the organic phosphorus of the casein. The amount of residue obtained, therefore, will depend not only upon the total amount of inorganic material in the casein but upon their relative amounts. Thus, if there is not sufficient lime present to combine with all the organic phosphorus, some of this latter will be lost during ignition. In order to prevent any such loss, it is usual to add a small quantity of calcium acetate before the ignition and then to subtract from the final weight the equivalent amount of calcium oxide to that of the calcium acetate added. The result of this determination is known as the " total ash content ", whilst that obtained by simple incineration is known as the " proximate ash content ".

Determination of total ash. About 2 g of the casein are weighed into a suitable dish, 5 ml of a solution of calcium acetate equivalent to 30 g of CaO per litre are added, and the mixture allowed to stand for 20 minutes. The contents of the dish are then dried and gently ignited until a white ash is obtained. From the weight obtained, 0·15 g is subtracted to allow for the calcium oxide. The following results obtained by Snyder and Hansen (1933) are typical—

Total ash	*Proximate ash*	*True ash**
3·50	1·92	1·89
4·17	2·71	2·56
3·45	2·73	1·84
3·55	2·99	1·94
3·37	3·20	1·76

* Total ash less 1·61, the equivalent, as P_2O_5, of the organic phosphorus in the ash.

Determination of moisture

The determination of moisture in casein is no less difficult than in other organic materials. Too thorough drying may cause decomposition, whilst too little drying will give low results. Distillation with xylene normally gives good results, or the heating may be carried out *in vacuo* at 70° to 80°. Where much free acid is present, decomposition during heating at 100° is probable, but by taking frequent weighings and observing the loss in weight, the loss due to decomposition, which will be fairly constant in a given time, may be distinguished from loss due to moisture. The amount of moisture should not exceed 10 per cent; it is usually much less.

Determination of fat

Direct extraction with ether in a Soxhlet tube, even after grinding with sand, is inclined to give low results. It is generally conceded that a modification of either the Werner-Schmid or the Röse-Gottlieb method is satisfactory, although there is some difference of opinion as to which is the better. The former may be carried out as follows.

Five grams of casein are intimately mixed with 10 ml of water, and 20 ml of concentrated hydrochloric acid are added, after which the mixture is heated in the boiling water-bath for forty minutes. After cooling, the fat is extracted with ether in the usual way, the dried residue being re-extracted with petroleum spirit in order to correct for any non-fatty solids extracted by the wet ether.

Determination of acidity

It is difficult to make this determination; in fact, different results will be obtained by different methods. A simple direct titration yields the *free acidity*, but solution in standard alkali and back-titration will yield the *total acidity*, the figure for which, in general, will be quite different from the figure for free acidity. For control work the titration may be carried out as follows. One gram of casein is dissolved in 25 ml of 0·1 N sodium hydroxide solution and titrated back with 0·1 N hydrochloric acid to phenolphthalein. The acidity is expressed as ml 0·1 N alkali required for one gram of casein. The U.S.A. specifications require the calculation to be made on the dry, fat-free and ash-free substance, when a figure of not more than 10·5 should be obtained.

Nitrogen. This is determined by the Kjeldahl process in the usual way.

Lactose. Lactose may be extracted by shaking 10 g for four hours with 250 ml of 50 per cent alcohol. The lactose is determined on an aliquot portion of this solution in the ordinary way.

Other tests. Casein is also tested for cleanliness (by solution in sodium hydroxide solution), colour, odour, solubility, and strength. The methods available are not sufficiently definite for them to be standardised, although many of them are excellent for comparison purposes in the same laboratory.

Determination of phosphates and glycerophosphates

Five grams are transferred to a stoppered cylinder containing 40 ml of warm distilled water, and alkali is added little by little to dissolve the casein until a distinct colour is shown to phenolphthalein (shaking well till dissolved completely); then the solution is cooled to 15° C and made up to exactly 99 ml. Four ml of acid mercuric nitrate solution (p. 370) are added, and the whole is shaken with much vigour till the precipitate is in a fine state of division, and then filtered.

To 20 ml, 20 ml of molybdate solution[1] are added, and the mixture allowed to stand cold for three hours. After filtering into a Gooch crucible and washing by decantation four times with 2 per cent nitric acid, and then six times with 10 ml of 1 per cent potassium nitrate, the seventh washing is tested, and it must not require more than 2 drops 0·1 N NaOH to give a pink colour to phenolphthalein; if still above this limit of acidity, the washing is continued till it is attained. The contents of the Gooch crucible are washed back into the precipitating vessel, using about 50 ml of water, raised to the boil, 1 ml of 0·5 per cent phenolphthalein is added, and the solution titrated hot to a faint pink with 0·1 N caustic soda. Then—

$$\text{ml } 0{\cdot}1 \text{ N NaOH required} \times 0{\cdot}0313 = P_2O_5 \text{ as phosphates}$$

To another 20 ml in a porcelain or silica basin, lime (free from phosphates) is added until pink; the mixture is evaporated to dryness and ignited cautiously; the ash is dissolved in strong nitric acid, again evaporated, and dissolved in dilute nitric acid with the addition of about 50 ml of water and 50 ml of molybdate solution, warmed to 40°; it is allowed to stand for fifteen minutes and then filtered on a Gooch crucible, washing as before, but titrating with N caustic soda. Thus—

$$\text{ml N NaOH required} \times 0{\cdot}95 = Na_2\overline{Gl}PO_4$$

If the ash of 1 g is dissolved in strong nitric acid and treated exactly as above, the difference between the titration of this molybdate precipitate and that obtained above, expressed as ml N NaOH × 0·313, will give the P_2O_5 as organic phosphate, and this multiplied by 51·3 will give an estimate of the casein.

Determination of solubility

To 1 g in a test-tube add 15 ml of water, shake well, warm to 50° C, and shake again. A soluble casein should dissolve. If it does not, add 0·125 g of sodium carbonate to 5 g, mix well, and test solubility; a good casein should now dissolve.

Before submitting to the above test, samples of casein should be ground to a fine powder.

The determination of the solubility of acid caseins in a solution of borax is employed as a guide to the ease of solubility of the casein. Several modifications of the process have been suggested; that due to Mummery and Bishop (1930) is as follows.

REAGENTS. Borax solution—20·833 g of $Na_2B_4O_7,10H_2O$ dissolved in water and made up to 1 litre.

Bromthymol-blue indicator—One decigram of the powder macerated in a mortar with 3·2 ml of 0·05 N sodium hydroxide solution and made up to 250 ml with distilled water.

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[1] *Ammonium molybdate solution*—Mix 14 ml of strong ammonia (sp. gr. 0·880) with 28 ml of water; add 10 g of molybdic acid and stir until all is dissolved. Add this solution, slowly and with constant stirring, to 125 ml of nitric acid (sp. gr. 1·2); stand the solution in a warm place for a few days and decant the clear liquid for use. A slight deposit may form on keeping.

Standard buffer solution—Made according to Clark and Lubs' (1916) formula, with potassium di-hydrogen phosphate and caustic soda.

Distilled water, pH 6·0.—For this determination, it is necessary to work with distilled water of a constant pH value. It is found that freshly-boiled distilled water, pH 7·0, gradually absorbs carbon dioxide, with a consequent fall in pH. A point of equilibrium is attained in the vicinity of pH 6·0; very slight adjustment is necessary when the pH value of the water is above or below this figure.

METHOD. Two grams of lactic acid casein, ground if necessary to pass a 30-mesh sieve (opening 0·0223 in.), are placed in a ¾-in. test-tube, 12 ml of borax solution are added, and the test-tube placed in a water-bath at 70° C. The casein is stirred thoroughly and at frequent intervals for 45 min. If the casein is not fully dissolved after 45 min., incomplete solubility is demonstrated.

When the casein is dissolved, the solution is made up to 100 ml with distilled water, and 1 ml is pipetted into another test-tube, together with 10 ml of distilled water. To the diluted solution of casein in borax are added 5 drops of bromthymol-blue indicator, and the colour is compared with that of the standard buffer solution. The requisite turbidity of the buffer solution may be produced with a few drops of a suspension of colloidal silica, or a comparator can be employed.

The pH of the solution after dilution, which Mummery and Bishop term the " solubility index ", indicates the ease of solubility of the casein.

Solubility index	*pH value*	*Colour*
Very good	Above 6·8	Blue
Good	6·8 to 6·4	Green
Passable	Below 6·4	Yellow

These workers found that caseins which do not completely dissolve when submitted to the above procedure give a pH value of 6, or lower if the undissolved particles consist of casein; in some cases, however, where the undissolved particles proved to consist of albumin, the pH value was 6·4 or higher.

Determination of casein in milk chocolate, etc.

Baier and Neumann (1909) estimated casein in solid preparations, and especially in milk chocolate, as follows.

Ten grams of the fat-free substance are rubbed up in a mortar with a 1 per cent sodium oxalate solution, and the paste washed into a 250-ml flask with about 200 ml of the sodium oxalate, the solution heated to boiling, and made up approximately to volume with hot oxalate solution. After standing for eighteen to twenty-four hours, being shaken at intervals, the solution is made up to volume, and 100 ml of the mixed filtrate are treated with 5 ml of 5 per cent uranium acetate solution, and 33 per cent acetic acid added, drop by drop, till the casein separates; the precipitate is centrifuged or filtered, washed with water containing 5 per cent of uranium acetate solution and 3 per cent of acetic acid solution per 100 ml, the nitrogen estimated by the Kjeldahl method, and the result multiplied by 6·38.

The following formulae will give approximations to the amount of milk constituents, calculated from the casein present in preparations such as milk chocolate—

$$\left.\begin{array}{l}\text{Casein} \times 1{\cdot}14 = \text{total proteins}\\ \quad\;\;,, \quad \times 1{\cdot}56 = \text{milk-sugar}\\ \quad\;\;,, \quad \times 0{\cdot}25 = \text{ash}\\ \quad\;\;,, \quad \times 2{\cdot}95 = \text{solids-not-fat}\end{array}\right\}\ \text{of milk solids}$$

The A.O.A.C. (1950) have suggested the following method for the determination of casein in milk chocolate. Weigh 10 g of the finely-divided sample, not necessarily defatted, into a 500-ml conical flask and add 250 ml of a 1 per cent solution of sodium oxalate. Boil gently for a few minutes, cool, add 5 g of magnesium carbonate and filter. Determine the nitrogen in 50 ml of the filtrate. Pipette 100 ml of the filtrate into a 200-ml calibrated flask and dilute with water almost to the mark. Precipitate the casein by adding 2 ml of glacial acetic acid or 1 ml of sulphuric acid, make to volume, shake, filter and determine the nitrogen in 100 ml of the filtrate. The difference between the two nitrogen determinations gives the nitrogen derived from the casein which, multiplied by 6·38, gives the weight of casein in 2 g of the sample.

Specification for casein

The U.S.A. Government Specification for casein issued in 1919 contained the following provisions—

Colour: White or light cream.
Odour: Nearly odourless with not more than a trace of sourness.
Moisture: Not more than 10·0 per cent.
Fat: Not more than 1·0 per cent (on water-free basis).
Ash: Not more than 4·0 per cent (on water-free basis).
Nitrogen: Not less than 14·25 per cent (on water-, fat-, and ash-free basis).
Acidity: Not more than 10·5 ml of 0·1 N alkali per gram.

Results of analysis

The following results for the analysis of commercial casein have been obtained—

Table 22.6—Constituents of commercial casein

Type of casein	Water	Fat	Ash	Acidity	Authority
Lactic acid	6·2 to 9·8	0·2 to 1·4	2·1 to 5·4	5·1 to 12·5	Browne
Hydrochloric acid . .	4·9 ,, 8·6	0·0 ,, 1·2	3·1 ,, 6·1	6·7 ,, 10·7	,,
Rennet	5·5 ,, 10·3	0·4 ,, 0·8	7·2 ,, 8·4	5·5 ,, 11·3	,,
Acid casein . .	1·7 ,, 9·6	0·01 ,, 0·1	0·0 ,, 0·5	. .	Richmond
Rennet	0·6 ,, 10·9	0·1 ,, 0·6	5·0 ,, 8·6	—	,,

CHAPTER 23

THE ANALYSIS OF BUTTER AND BUTTER FAT

(1) THE ANALYSIS OF BUTTER

Butter must legally contain not more than 16 per cent of water. This is the only legal standard for a constituent, but it is laid down by the Ministry of Food that butter shall not contain more than 2 per cent of salt. The usual data to be determined in the analysis of butter are water, solids-not-fat, fat, salt, and preservatives. It is also occasionally of interest to determine the actual curd, or the casein. The methods indicated below may also be used in the analysis of margarine.

Sampling

The most important datum is the percentage of water. As the water is not always equally distributed throughout the mass of butter, especially in butters which have been salted, it is necessary to take precautions to obtain a fair sample—a matter of some difficulty. It is not advisable to use a scoop, as water is liable to be squeezed out while forcing it into the lump. Perhaps the fairest way of sampling is to cut the lump into halves, and to take a piece near (not at) one top corner, a second piece in the middle, and a third near the opposite bottom corner. The three pieces should be placed in a wide-mouthed stoppered bottle, melted at as low a temperature as possible, and shaken violently till the mass is nearly solid. If the analysis is to be commenced at once, suitable quantities may be poured out while the butter is still in a semi-liquid condition, and weighed as soon as possible. The water by this means is equally distributed throughout the sample, and a small quantity will be representative of the whole sample. With well-made fresh butter the differences in the distribution of water are small, and a single sample taken from any part of the lump will represent with fair accuracy the whole bulk. Where extreme accuracy is not required, the melting and shaking of samples of fresh butter may be omitted.

The water in butter may also be uniformly distributed by warming to such a temperature that the butter begins to lose its consistency, and stirring vigorously with a stout glass rod. The mixing of salt butter should not be omitted if accuracy is a desideratum.

Determination of water

The following methods are used for the determination of the water.

(1) About 10 or 20 g are weighed out into a small porcelain basin provided with a glass stirrer. This is placed over a very small flame, or on a sand-bath, and the butter carefully but vigorously stirred till all signs of frothing cease.

The temperature must be so regulated that spurting is avoided, and that the "curd" does not become appreciably browned by the heat. The basin and its contents are, after cooling, weighed; the loss of weight indicates water.

(2) A basin is filled with pumice, which is broken in pieces about the size of a small pea, washed and ignited; 2 or 3 g of well-mixed butter are weighed in, and the basin placed in a drying oven at 100° C (212° F), through which a good draught passes. At the expiration of an hour the basin is cooled and weighed, and then replaced in the oven for a further half-hour; weighings are made at the expiration of succeeding half-hours till the weight ceases to diminish. The lowest weight obtained is taken as that of the dry butter. The difference between this weight and that of the original butter is taken as water.

(3) Four to five grams of butter are weighed into a wide-mouthed flat-bottomed conical flask, which is placed in a water-oven and shaken every ten minutes for the first half-hour, after which it is shaken every half-hour. At the expiration of four hours it is cooled, weighed, and returned to the bath for another hour; if there be any loss, the drying is continued till an hour's drying does not cause any diminution of weight.

(4) From 2 to 2½ g of well-mixed butter are weighed into a flat-bottomed basin about 2¾ inches diameter. This is placed in the water-oven till just melted, and 1 to 1½ ml of strong alcohol are added; the basin is replaced in the water-oven, and weighed after two hours. The loss represents water.

(5) About 2 g of butter are weighed into a flat-bottomed metal dish about 2¾ inches diameter (platinum, aluminium or German silver are suitable metals). The dish and contents are dried in an oven maintained at 98° to 100° C for 3 hours. Cool in a desiccator and weigh. Replace in the oven for a further half-hour and weigh again.

Of the five methods, the first is the most expeditious and is nearly as accurate as the others; the second is the most accurate; the third is the most convenient if solids-not-fat and salt are also estimated; while the fourth and fifth are fairly accurate and require no attention. No one method has, however, any great advantage over the others. For routine testing, methods No. 1 and No. 5 are probably the most convenient, No. 1 being used when curd and salt are also estimated.

Dean and Stark volumetric process

This method, in one or other of its modifications, has been generally adopted for moisture determinations in cases where other volatile constituents or decomposition products besides water are present. The process depends on distilling the sample with a solvent immiscible with water (such as toluene, xylene or commercial heptane) and measuring the moisture which comes over in a specially constructed and calibrated receiver. A description of the method as modified by Tate and Warren (1936) is appended.

The apparatus conforms to the dimensions given in the accompanying sketch (Fig. 7).

Ground-glass joints are used, and commercial heptane is used as the entrainer.

Before use the whole apparatus is cleaned with chromic sulphuric acid, washed with water, then with acetone, and finally dried in a stream of dry air. An amount of the sample which will give as nearly as possible the maximum amount of water which can be measured (2 ml) is placed in the distillation

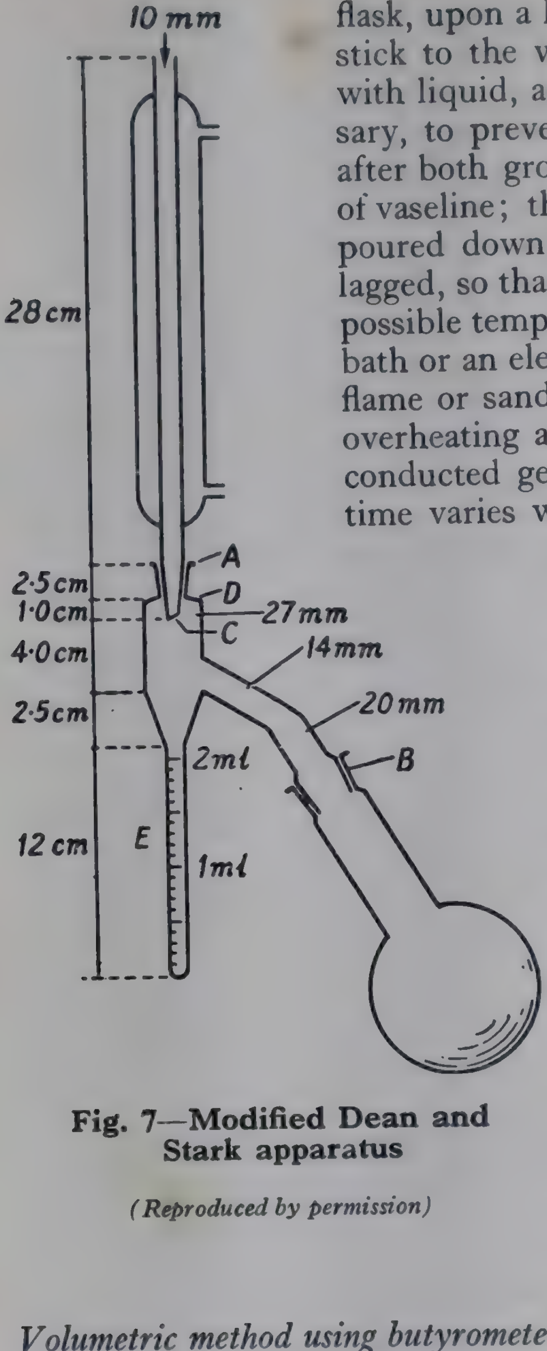

Fig. 7—Modified Dean and Stark apparatus

(Reproduced by permission)

flask, upon a layer of dry sand if it tends to cake or to stick to the walls of the flask. The sample is covered with liquid, and a few silica beads are added, if necessary, to prevent bumping. The apparatus is assembled after both ground joints have been treated with a trace of vaseline; the collecting tube *E* is then filled with liquid poured down the condenser. The whole apparatus is lagged, so that the heating bath can be kept at the lowest possible temperature. Heating is best effected by an oil-bath or an electrically heated air bath, since use of a free flame or sand or asbestos baths generally leads to local overheating and possible decomposition. Distillation is conducted gently for as long as may be necessary; the time varies with the substance under examination, and can only be determined by trial. At the finish the bulk of the water will be found in the graduated tube, but there are always some water-drops adhering to the inside of the condenser. These are readily removed by a spray of entraining liquid. A suitable spray is made by sealing the end of a piece of glass tubing, and piercing four small holes near to the seal. With this a horizontal spray can be projected with considerable force on to the drops. The condenser is then removed, and any water-drops adhering to the walls of the cell are swept down with a camel-hair brush moistened with the entraining liquid.

Richmond (1920) has described a similar process due to Gray, the entrainer in this case being a mixture of amyl acetate and amyl valerate.

Volumetric method using butyrometer

Water in butter, margarine, etc., may also be determined by a special form of butyrometer similar to a Stokes tube. It consists of an elongated bulb 5 ml in capacity, connected by a graduated tube with a vessel in which a cork carrying a cup of 3 ml capacity is inserted. The only reagent necessary is diluted sulphuric acid, made by diluting commercial sulphuric acid (sp. gr. 1·820 to 1·825) with an equal bulk of water.

Five ml of diluted sulphuric acid are measured into the butyrometer, which is placed open in the machine, and whirled for about two minutes, in order to bring all the liquid into the bulb; the level of the acid (at as near 60° F (15·5° C) as possible) is read off on the graduated scale. About 2½ to 3 g of butter are weighed into the cup, and after the cork has been inserted, the butyrometer is placed in a water-bath maintained at 60° to 70° C (say 150° to 160° F) to melt the butter; when this has been accomplished, the whole is shaken well till the contents form a uniform emulsion; after standing for a minute in the water-bath, the butyrometer is placed in the machine and whirled three times, warming in the water-bath for about two minutes between each; after the third whirling, it is cooled to as near 60° F as possible, and the level of the aqueous liquid where it joins the fatty layer is read off. The difference between this reading and the level of the acid will give the percentage of water if exactly 3 g of butter have been taken; should any other weight have been taken, it is necessary to multiply the result by 3 and divide by the weight taken; thus, in an experiment 2·780 g of butter were taken, the level of the acid was 2·5° and the level of the aqueous liquid 14·5°; the percentage of water indicated is, therefore,

$$\frac{(14{\cdot}5 - 2{\cdot}5) \times 3}{2{\cdot}780} = \frac{12 \times 3}{2{\cdot}780} = 12{\cdot}95 \text{ per cent}$$

Determination of solids-not-fat and salt

Heat gently the residue from the determination of moisture by methods (1) or (3) above in order to melt the fat, stir in about 30 ml of petroleum ether (or dry methylated ether), allow to settle, and decant through a tared filter-paper in such a manner as to retain most of the non-fatty residue in the original receptacle. Treat the residue in the dish 3 or 4 times with 10 ml of petroleum ether, decanting through the filter as before, and finally wash the lip and outside of the dish and the edges of the filter with a stream of petroleum ether from a wash-bottle until the filtrate is free from fat. The dish and rod or flask and the filter-paper are dried at 100° C until constant in weight. The combined increase in weight of the empty dish or flask and the filter-paper represents the weight of curd and salt in the weight of sample taken.

To determine the salt, the residue is treated with hot water and filtered; the filter, together with the residue, washed; and the filtrate, or an aliquot portion of it, is titrated with standard silver nitrate solution, using potassium chromate as indicator. It is essential that the solution should be cold before titration, and the silver nitrate solution should be standardised on pure sodium chloride. From the amount of silver nitrate solution used the weight of salt is readily calculated. It is convenient to make the silver nitrate solution of such strength that

1 ml = 0·005 g of sodium chloride
or 1 litre contains 14·520 g of silver nitrate

The titration may also be carried out by Volhard's method, in which a definite excess of silver nitrate solution is measured into the solution to be titrated and the excess is back-titrated with standard potassium thiocyanate solution, after adding 10 ml of a saturated solution of iron alum in 10 per cent nitric acid as indicator.

Arup (1929c) has suggested a rapid routine method which gives results which approximate very closely to the standard method. Weigh about 3 g of the sample in a chloride-free filter-paper or cigarette paper and drop the whole into a 150-ml conical flask. Add 10 ml of boiling distilled water, shake well, and titrate with 0·1 N silver nitrate solution, using chromate indicator; towards the end-point, close the flask and shake well between each addition of silver nitrate.

Determination of ash

In place of a determination of the salt, an ash determination is often made, and the ash taken as salt. The results are, however, always slightly above those obtained by titration, as butter itself, to which no salt has been added, gives a small ash; preservatives, such as borax, will also increase the weight of the ash.

Solids-not-fat. The weight of salt found by titration is subtracted from that of the residue left after the extraction of the fat; the difference represents the solids-not-fat.

Curd. This can be determined by submitting the residue, left after extraction of the fat, to Kjeldahl's process for the determination of nitrogen (p. 366), and multiplying the nitrogen found by 6·38.

Milk-sugar. This may be determined on a portion of the solution used for the titration of salt by Lane and Eynon's Fehling solution method (p. 375).

Dried milk is occasionally added to butter, and may be detected by determination of the milk sugar, which does not exceed 0·4 per cent in a normal butter.

The determinations of curd and milk-sugar are very rarely required.

Determination of fat

This is usually arrived at indirectly by subtracting the percentages of moisture and solids-not-fat (including salt) from 100. If desired, a direct determination may be made by filtering the petroleum ether solution of the fat in the estimation of solids-not-fat and salt into a tared flask. The ether is distilled off, the residue dried at 100° C with the usual precautions to remove ether vapours, and weighed.

For routine purposes, an approximate value of the fat content of butter or margarine can be obtained by filling flat-bottomed 6-in. test-tubes by gently heating the top of the tube and forcing pieces of the sample in by means of a spatula. The contents of the tubes are melted completely by standing in a beaker of hot water on a water-bath, the tubes centrifuged, and the total length of the column of sample and the length of the column of fat read off by means of a ruler held alongside the tube. The percentage of fat by volume can then be calculated.

Detection of preservatives

These substances, with the exception of salt, are now illegal in butter and are, therefore, rarely used. Previously the most frequently used preservatives were compounds of boron; sulphites and nitrates have also been used, usually in conjunction with borates; fluorides have also been employed; formalin has been recommended, but appears to be rarely used. These should be tested for in the aqueous portion which sinks to the bottom on melting the butter at a low temperature. The reaction with turmeric-paper applied to the liquid direct will show the presence of free boric acid. If no reaction or a feeble one be obtained, a little of the liquid may be acidified with very dilute hydrochloric acid, and tested with turmeric-paper. A pinkish-brown coloration, turned greenish-black by dilute alkali, will show the presence of boric acid in combination. It will usually be found, if the butter is preserved in this way, that a reaction is obtained from the liquid itself, and a much stronger one after acidifying.

The presence of sulphites may almost always be detected by the smell of sulphurous acid developed on acidifying. Nitrates may be found by the diphenylamine test. Monier-Williams tests for fluorine by shaking 10 g, after melting, with ether and 1 or 2 ml of water in a separating-funnel. The aqueous layer is run off into a test-tube, a few drops of hydrogen peroxide added, and 1 ml of a solution containing 2 per cent of titanium sulphate in 10 per cent sulphuric acid. The colour is compared with a similar test made on pure butter (or margarine). In the presence of fluorides the orange-yellow colour of the peroxide will be partially or wholly discharged. While not quite characteristic, it is a useful sorting test, and the presence of fluorides may be proved by testing as in milk (p. 403).

For the quantitative determination of preservatives, 50 g of butter should be placed in a stoppered cylinder, 50 ml of chloroform added, and the mixture warmed gently till perfect mixture takes place. A quantity of water which will, with that present in the butter, make up 50 g is added, and, after shaking, the cylinder is put aside to allow the aqueous portion to separate. Each millilitre of the solution will contain the preservative in 1 g of butter.

For the determination of boric acid, Thomson's method is convenient (p. 394). As butter is practically free from phosphates, the process for their removal may be omitted, and the titration performed on an aliquot portion of the solution which has been made alkaline, evaporated to dryness, and ignited; the ash is extracted with hot water, and titrated first with acid till neutral to methyl-orange, and then with alkali in the presence of glycerol, till neutral to phenolphthalein; the result will be the total boric acid, free and combined.

Alternatively, the modification of Thomson's process used in the Government Laboratory for fatty foods may be used. This method is described on p. 457.

Richmond and Harrison devised a rapid method for the estimation of boric acid in butter. Twenty-five g of butter are weighed into a beaker and just melted in the water-oven; 25 ml of water are added, and the contents of the beaker mixed well by stirring; the aqueous portion is allowed to settle, the contents are again mixed, and allowed to settle. Twenty ml of the lower layer are withdrawn, and the boric acid estimated therein by the method of Richmond and Miller given on p. 395. The weight of boric acid multiplied by $\frac{100 + W}{20}$ (W = percentage of water) will give the percentage of boric acid.

The following rapid method of determination is satisfactory for routine purposes. Weigh 6·2 g of sample into a 250-ml wide-mouth flask. Add 50 ml water, a few drops of methyl orange indicator solution, acidify with 0·1 N hydrochloric acid and boil for a few minutes. Cool, and neutralise with 0·1 N sodium hydroxide to methyl orange. Add 20 ml of glycerine, or 2 g of mannitol, and titrate with 0·1 N sodium hydroxide to phenolphthalein. Each millilitre of 0·1 N sodium hydroxide is then equivalent to 0·1 per cent of boric acid in the sample.

Other preservatives may be detected and determined on aliquots of the aqueous solution prepared from 50 g of the sample (above), using the methods described under preservatives in milk.

Methods for the examination of butter fat are described in the following sections of this chapter.

Other standardised tests for butter are described in the British Standard *Methods for the Chemical Analysis of Butter*, No. 769—1952, and in *A.O.A.C.* (1950).

(2) THE PHYSICAL EXAMINATION OF BUTTER FAT

Determination of density

The density of butter is best determined by the pycnometer. This is first filled with distilled water, and the weight of the water which it holds at 37·8° C determined. After drying, by placing in the water-oven and drawing a current of air through it, it is filled with the fat and placed in water at 37·8° till the volume is constant; the temperature must be accurate to 0·1° if the result is required to be exact to the fourth place of decimals. The weight of fat divided by the weight of water will give the density at 37·8°/37·8°.

The Westphal balance may be employed; the apparent density of water at 37·8° must be determined, and the density of fat indicated by the instrument divided by this to obtain the density at 37·8°/37·8°.

The density is also sometimes determined by a hydrometer. If this instrument be used, it should be tested in fats of known density, and its indications thus controlled. A. Meyer states that the height of the meniscus depends somewhat on the barometric pressure, but the error due to this cause is not likely to exceed the experimental error of reading. Should the temperature not be exactly 37·8° C, a correction of 0·0007 for each degree may be added for temperatures above and subtracted for temperatures below, 37·8° C.

If it be desired to take apparent densities at 100°/15·5° in glass, the instrument should be standardised at 15·5°, and the density determined as above.

Richmond used a bulb of specific gravity 0·865 at 15·5° for the purpose of determining rapidly an approximate density. A test-tube is filled with the fat, the bulb dropped in, and the tube placed in boiling water. If the bulb floats at the top, the density is above 0·865; if it sinks, the density is below. This has proved a fairly good rough test.

Determination of refractive index

When light passes from one medium to another it passes in a straight line only when it falls perpendicular to the surface separating the two media. If it passes through at an angle to the surface, it is bent or refracted, and the ratio of the sine of the angle made by the path of the ray with the perpendicular to the surface in the first medium to the sine of the angle made by the path in the second medium with the perpendicular is a constant, known as the *index of refraction*. As the sine of an angle of 90° is 1, it is seen that the ratio between the sine of the angle at which light is first reflected and 1 is the index of refraction; this angle is termed the *angle of total reflection*. As it is more convenient to measure this than to measure the two angles and deduce the ratio of the sines, in practice the angle of total reflection is frequently measured.

Müller was the first to apply the determination of the refractive index to the analysis of butter. He allowed the butter to solidify slowly, absorbed the liquid portion with filter paper, extracted this with ether, and examined it in the Abbé refractometer, an instrument which measures the angle of total reflection. Skalweit examined this method and showed that it was important to operate at a fixed temperature.

The butyro-refractometer

The Zeiss butyro-refractometer measures the angle of total reflection and is a modification of the well-known Abbé refractometer. It consists of two prisms of glass, hinged so that they can be separated. The light enters at the bottom, passes through the prisms, and is viewed through a telescope having a fixed scale in the focus of the eye-piece. The prisms are provided with a jacket through which water, the temperature of which is indicated by a thermometer, is passed. A drop of the filtered fat is placed on the glass surface of the lower prism, spread over it, and the prism closed; the reflector is so adjusted as to reflect clear daylight or lamplight through the prisms, and the refractive index in scale degrees is read off.

This instrument is extremely rapid, as a determination, including reading of the temperature and scale degrees, does not take more than a minute. After use, the instrument should be cleaned by rubbing off the fat with a duster, and polishing the prisms with a clean linen cloth slightly moistened with alcohol.

The connection between x and $[n]_D$ is expressed by the formula

$$287{\cdot}7 - x = 839{\cdot}4 \sqrt{1{\cdot}5395 - [n]_D}$$

This can be easily worked out with a table of four-figure logarithms. This formula was simplified by Richmond from those of Roberts and Liverseege.

There is a difference in the refractive index depending on the light used; this is corrected in the instrument by making the prisms of different kinds of glass, so that when used with butter, ordinary white light behaves as if it were simple light. Other fats (and adulterated butters) may be tinged at the edge with blue or red. In this case it is not easy to read the dividing-line accurately. Richmond and other workers used the sodium flame, obtained by heating sodium chloride in a Bunsen burner, as the source of light, and found that absolutely sharp readings can thus always be obtained. The readings with butters do not differ, whether white light or sodium light be used.

The refraction figure varies by 0·55 scale degree for each 1° C, and can be corrected by means of this factor if the temperature differs from that adopted as normal.

For the correction of scale readings taken at one temperature to any other temperature or to that adopted as a standard, Leach and Lythgoe devised a slide rule, but, except for small differences of temperature, Richmond found that the readings were not strictly correct.

A chart for the correction of butyro-refractometer readings for temperature may be constructed thus. Select a sheet of squared paper at least 120 units by 200 units wide; at a point 34 units from the bottom, set out horizontally a series of points 5 units apart, and at a point 119 units from the bottom a similar series of points 7 units apart; join corresponding pairs of points to form a series of temperature-lines. The middle vertical line is selected as the standard temperature, say 35°, and each line to the right will represent a temperature of 1° higher, and to the left 1° lower, than the next preceding line.

From the bottom, at a point 100 units from the standard temperature-line to the left, draw a line to the point which lies 20 units from the bottom and 100 units to the right of the standard temperature-line; this will represent 0°

on the scale of the butyrometer; draw parallel to this a series of lines 10 units apart, measured vertically, and mark these 10°, 20°, etc., of the refractometer scale. To use the chart, find the point of intersection of the lines corresponding to the observed temperature and scale lines (differences between the lines can be estimated with sufficient accuracy by the eye); the distance measured horizontally between this point and the vertical standard line will give the correction to be added if on the right, or subtracted if on the left (10 units of distance equal one scale degree of correction).

This chart is easy to make and still easier to use, and Richmond found it to give very accurate results over a considerable range of temperature, not only for butter, but also for other oils and fats, and for the standard fluid.

The results given on p. 239 were determined at a temperature of 40° C. For the purpose of checking the instrument, a standard fluid (Normal Flüssigkeit) is supplied, and the scale should be verified from time to time by its use. The point at which the dividing-line should lie at 35° C is marked on the instrument, and the scale should be brought to this point by means of a key just above the prisms.

The Abbé refractometer

This instrument can be employed to obtain the refractive index of any fluid, whether an oil or an aqueous solution, and it requires only a few drops of the liquid for an accurate determination. The instrument is similar in appearance to the butyro-refractometer, the essential differences being in the provision of a side arm bearing a scale, calibrated directly in refractive indices; a device consisting of two compensating prisms which enable the band of colour which appears at the border-line, when daylight or ordinary artificial light is used, to be completely eliminated; and the fact that the instrument covers a very wide range of refractive indices from 1·3 to 1·7. Readings are possible to the fourth decimal place; therefore, to obtain the highest degree of accuracy, it is important that the temperature of the prisms should be accurately controlled by means of a constant stream of water which can be readily maintained at the desired temperature.

In order to make a determination, the catch holding the two faces of the prisms together is slackened off, but not necessarily opened completely, and a few drops of the liquid are allowed to run down the funnel-shaped channel at the side of the prisms. On tightening the catch, the liquid is evenly distributed over the whole space between the two prisms. The mirror is then adjusted, the telescope moved until the border-line of the shadow appears on the cross-lines of the telescope field. The border-line is finally adjusted to remove colour by means of the compensating prisms, and the reading is taken through the lens placed over the scale.

In order to check the instrument, the makers supply a testing-plate and they also give instructions for its use. As these are somewhat complicated it is simpler to use a standard fluid such as mono-bromonaphthalene, specially prepared for the purpose, which has a refractive index of 1·658 at 15° C.

The instrument may also be checked by taking the refractive index of distilled water, which should have the values given in Table 23.1.

If the scale setting is found to be inaccurate, it may be adjusted by turning the black milled ring until the border-line of the shadow coincides with the cross-lines when the scale is set at the correct refractive index of the test liquid between the prisms at the temperature of the determination.

Table 23.1—Index of refraction of distilled water (*Wagner*)

Temperature (°C)	Index of refraction	Temperature (°C)	Index of refraction
30	1·3320	22	1·3328
29	1·3321	21	1·3329
28	1·3322	20	1·3330
27	1·3323	19	1·3331
26	1·3324	18	1·3332
25	1·3325	17	1·3332
24	1·3326	16	1·3333
23	1·3327	15	1·3334

Microscopic examination of butter in polarised light

This method is founded on the fact that when a crystalline substance is placed between two crossed Nicol prisms the light undergoes rotatory polarisation; the rays that would normally vibrate in the plane which would cause total reflection are caused to vibrate in a plane inclined to this, and the light consequently passes through the second Nicol prism. Substances which have no crystalline structure do not cause any interference with the plane of vibrations.

This method was first applied by Campbell Brown to detect adulteration of butter with foreign fat. The fat of milk when churned into butter is devoid of crystalline structure. The fats of which margarine is composed, having been melted and cooled, usually acquire a more or less pronounced crystalline form.

The method was later studied by Taylor, Pizzi, and others, and is fairly reliable. The following are sources of error. The presence of salt, salicylic acid, and other crystalline substances added to butter as preservatives, or accidentally mixed with it, will cause the light to pass, and may be mistaken for crystalline fat; but a simple microscopical examination will usually reveal the nature of particles of this kind, and an experienced observer will rarely be misled. Butter which has been melted, re-emulsified, and re-churned will behave to this test as margarine, though no similar appearance is noticed in butter which has been kept just below the melting-point for some length of time. Margarine which has been prepared by emulsifying the fat with skim-milk with a good emulsor, separating the cream, and churning this with ordinary cream, behaves as butter, and Pizzi succeeded in adding 30 per cent of foreign fat to butter in this way without being able to distinguish it. Finally, rancid butter, and butter which has been at once churned from pasteurised cream at a low temperature, may sometimes give an appearance resembling margarine. Butter prepared from clotted cream shows many crystalline particles.

It is apparent that this test must be used with reservations, but it is without doubt of use as corroborative evidence in cases where other analytical data are not absolutely conclusive.

The method is carried out as follows. The outer portions of a piece of butter are removed, and a piece about the size of a pin's head is transferred from the freshly exposed surface to a clean microscope slide. A cover glass is placed on the top, and the butter spread out by gentle pressure on the upper surface of the cover. The slide is placed on the stage of a microscope fitted with crossed Nicol prisms, and examined with a 1-inch objective or higher power. To exclude light from the upper surface, a blackened cardboard tube may be placed over the slide in such a manner that the objective dips into it, and the light falling on the upper portion of the slide is cut off. When pure butter is examined, the field is uniformly dark, and only with the greatest difficulty can any structure be distinguished. When margarine is present, certain portions of the field have a bright appearance, and indistinct crystalline forms can be made out. If any distinct and bright crystals are seen, the Nicol prisms should be turned parallel and the slide examined in that spot in order to see whether salt or other crystalline matter is present; there is not much difficulty in distinguishing this, owing to its great refractive power. The slide should be moved about to examine all parts of it, as, with small amounts of adulteration, the margarine is not distributed equally throughout; two or more portions from different parts of the sample should be examined.

As a check, a selenite plate (a crystalline form of calcium sulphate, which possesses the property of rotatory dispersion to a large extent) is next placed under the slide, the microscope focused, and the sample again examined. In this case the slide will be uniformly illuminated when the prisms are crossed, but will appear coloured, the colour depending on the thickness of the selenite and the position of the Nicol prisms; but when pure butter is examined the whole of the field appears of one colour. When margarine is under observation, certain parts of the field are seen to be of a different colour.

This modification is, when used by persons of absolutely normal vision, quite as delicate as the examination without selenite, but it cannot be generally recommended, as the perception of colour is a sense in which many persons—more than is commonly supposed—are deficient without necessarily being colour-blind. The usual colours which selenite plates are constructed to give, viz. red and green, are those which are least easily distinguished by the majority of those who suffer from weak colour-perception. It is advisable, therefore, never to omit the examination without a selenite plate.

It is of course essential to employ a good microscope, as any illumination of the slide, except by light which has passed through the polariser, will prevent the extinction of the field on crossing the Nicol prisms. Though it is impossible in practice to secure an absolutely dark field, this can be done with a good instrument and a cardboard tube over the slide with a near approach to completeness. Any marked illumination of the field when the Nicol prisms are crossed will greatly impair the delicacy of the test.

(3) THE CHEMICAL EXAMINATION OF BUTTER FAT

Preparation of the sample

A portion of the butter (about 20 g) is melted in a beaker and allowed to stand at a temperature of about 50° C until the water and curd have separated.

The fat is then decanted off through a dry filter-paper, placed in an oven maintained at about 50° to 60° C and refiltered if necessary. The fat should not be heated longer than is necessary to effect the above separation, but it should be remelted each time that a portion is taken for analysis.

After the butter has been melted and allowed to stand, and before it is filtered, the appearance of the fatty layer should be noted. If the butter be genuine, fresh, and well-made, the melted fat will usually appear transparent; while if it be mixed with butter substitutes, rancid, or churned at a high temperature, or if it has been melted and re-emulsified, the fat frequently has a turbid appearance.

DETERMINATION OF VOLATILE FATTY ACIDS

This is the principal method of examination of butter fat, and the process employed is known as the Reichert-Polenske-Kirschner process, which, as its name indicates, is a combination of three determinations. The modification of the method described in detail below is that standardised and recommended by the Analytical Methods Committee of the S.P.A. (1936b).

Reichert-Polenske-Kirschner process

This process does not determine the *total* quantities of volatile fatty acids, but only the amounts which distil over under certain carefully specified conditions; in order, therefore, to obtain strictly comparable results, these conditions should be rigidly adhered to.

REAGENTS

Glycerol.

Concentrated sodium hydroxide solution (50 per cent by weight)—Sodium hydroxide is dissolved in an equal weight of water and the solution is stored in a bottle protected from carbon dioxide. The clear portion free from deposit is used.

Dilute sulphuric acid solution—Approximately 25 ml of concentrated sulphuric acid are diluted to 1 litre, and adjusted until 40 ml neutralise 2 ml of the sodium hydroxide (50 per cent) solution.

Pumice powder—Pumice, ground, passing through a sieve B.S. No. 50,[1] and remaining on a sieve B.S. No. 90.

Phenolphthalein solution—0·5 g of phenolphthalein dissolved in 100 ml of industrial methylated spirit.

Alcohol—Industrial methylated spirit neutralised to phenolphthalein immediately before use.

Sodium hydroxide solution—Approximately 0·1 N solution of sodium hydroxide, of accurately determined strength.

Barium hydroxide solution—Approximately 0·1 N solution of barium hydroxide, of accurately determined strength.

Silver sulphate, powdered.

All reagents must be of the quality required for quantitative chemical analysis.

APPARATUS

One hundred-ml graduated cylinder, Class B, B.S. No. 604.

Fifty-ml pipette, complying with the N.P.L. Class B regulations.

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[1] See British Standard Specification for Test Sieves, No. 410—1931.

The assembly of the apparatus for the distillation is shown in Fig. 8, and details of the constituent parts are given below.

Flat-bottomed boiling-flask (Polenske)[1]—The flask shall be made of resistance glass and shall conform with the following details—

Volume contained to bottom of neck	310 ml	± 10 ml
Length of neck	75 mm	± 5 mm
Internal diameter of neck	21 ,,	± 1·0 ,,
Overall height	160 ,,	± 5 ,,
Diameter of base	45 ,,	± 5 ,,

Still-head[2]—The still-head shall be made of glass and shall conform with the dimensions given in Fig. 9.

Condenser—The condenser shall be made of glass and shall conform with the dimensions given in Fig. 8.

Receiver—The receiver shall be a flask with two graduation marks on the neck, one at 100 ml and the other at 110 ml, conforming with the British Standard Specification for Sugar Flasks, No. 675—1936.

Asbestos board[1]—An asbestos board, 120 mm diameter, 6 mm in thickness, with a circular hole about 65 mm diameter.

PROCEDURE

Weigh 5 g (tolerance not exceeding 0·01 g) of the fat into a Polenske flask. Add 20 g of glycerol and 2 ml of concentrated sodium hydroxide solution. (The burette containing the soda solution must be protected from carbon dioxide, and before withdrawal of the solution for tests, the nozzle must be wiped clean from carbonate, and the first few drops of solution rejected.) Heat over a naked flame with continuous mixing, until the fat, including drops adhering to the upper parts of the flask, is saponified, and the liquid becomes perfectly clear. Cover the mouth of the flask with a watch-glass.

[Make a blank test without fat, but using the same quantities of reagents and following the same procedure, and avoiding over-heating during the heating with soda (indicated by darkening of the solution).]

Measure 93 ml[3] of boiling distilled water, which has been vigorously boiled for 15 minutes, into a 100-ml graduated measuring cylinder. When the soap is sufficiently cool to permit addition of the water without loss, but before it has solidified, add the water to the flask, draining the cylinder for 5 sec., and dissolve the soap.

If the solution is not clear (indicating incomplete saponification), or is darker than light-yellow (indicating over-heating), the saponification must be repeated on a fresh sample of the fat.

Add 0·1 g of powdered pumice, followed by 50 ml of the dilute sulphuric acid solution, and connect the flask at once with the distilling apparatus shown in Fig. 8. Heat the flask, without boiling, until the insoluble acids are completely melted, then increase the flame and distil 110 ml in 19 to 21 minutes. The flow of water in the condenser must be sufficient to keep the temperature of the issuing distillate between 18° and 23° C. When the distillate reaches the 110-ml mark, remove the flame and replace the 110-ml flask by a cylinder of about 25 ml capacity to catch drainings. Close the 110-ml flask with a stopper, and, without mixing, place it in water at 15° C for 10 minutes so as to immerse the 110-ml mark. Remove the flask from the water, dry the outside, and invert the flask carefully, avoiding wetting of the stopper with the insoluble acids. Mix the distillate by four or five double inversions without violent shaking. Filter through a dry 9 cm No. 4 Whatman filter-paper (see Note 3, p. 497), which fits snugly into the funnel. Reject the first runnings and collect 100 ml in a dry flask; cork the flask, and retain the filtrate for titration as at *R*, below. The filtrate must be free from insoluble fatty acids.

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[1] During distillation the Polenske flask must fit snugly into the hole in the asbestos board so as to prevent the flame from impinging on the surface of the flask above the hole. A new asbestos board may be conveniently prepared by bevelling the edge of the hole, soaking in water, moulding the edge with a flask, and drying.

[2] The position of the side hole of the still-head relative to the stopper of the Polenske flask should also be as shown in the diagram.

[3] The temperature of the water in a cylinder rinsed out and refilled with boiling water is between 85° and 90° C; 90 ml of cold water correspond with 92·7 ml at this temperature, or, allowing 0·3 ml for water retained on draining, 93 ml altogether. Addition of the water hot avoids loss of time in dissolving the soap.

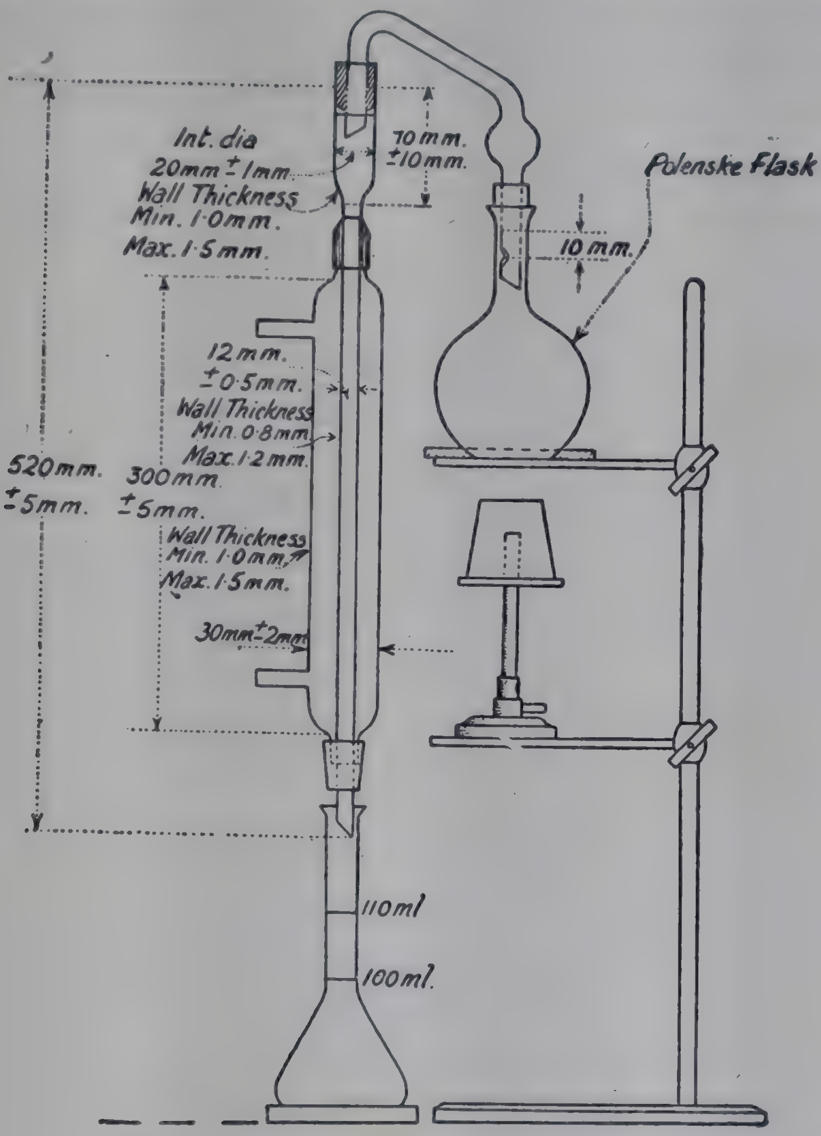

Fig. 8—Polenske distillation apparatus

(Reproduced by permission of the S.P.A.)

Detach the still-head and wash the condenser with three successive 15 ml portions of cold distilled water, passing each washing separately through the cylinder, the 110-ml flask and the filter, nearly filling the paper each time, and draining each washing before filtering the next. Discard the washings.

Dissolve the insoluble acids by three similar washings of the condenser, the cylinder and the filter with 15 ml of neutralised alcohol, collecting the solution in the 110-ml flask and draining the alcohol after each washing. Cork the flask and retain the solution for titration as at *P*, below.

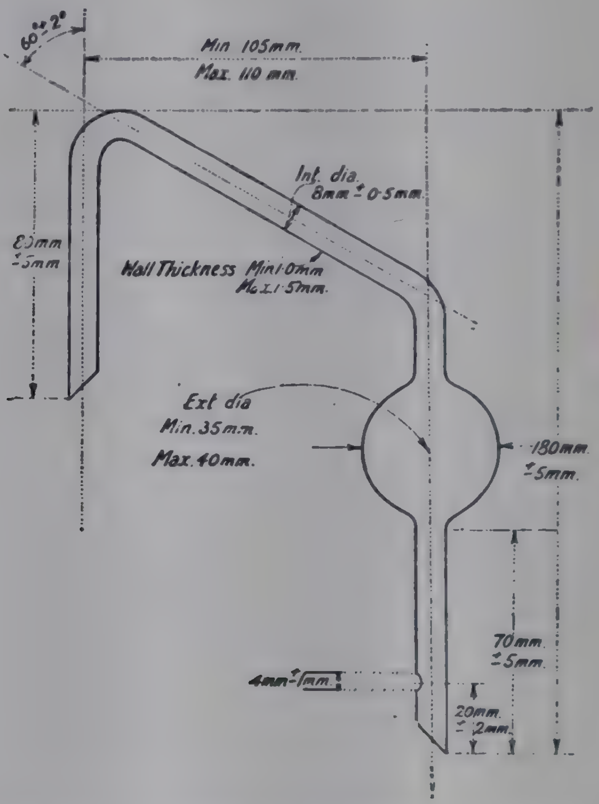

Fig. 9—Still-head for Polenske distillation apparatus

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(*R*)—Reichert[1] (or soluble volatile acid) value

Pour 100 ml of the filtrate containing the soluble volatile acids into a titration flask, add 0·1 ml of solution of phenolphthalein, and titrate with 0·1 N barium hydroxide solution[2] until the liquid becomes pink, rinsing the 100-ml flask with the nearly neutralised liquid towards the end of the titration.

[If the Kirschner value (see below) is to be obtained, the titration flask must be dry before use; note the actual volume of barium hydroxide solution used; drain the 100-ml flask into the titration flask, close with a cork, and continue as at *K*, below.]

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[1] It is considered that the name " Reichert " value is preferable to the cumbrous titles formed by associating (sometimes incorrectly) with Reichert's process the names of one or more of those who have modified it, such as Meissl, Wollny and Polenske.

[2] 0·1 N sodium hydroxide solution may be used for the titration if the Kirschner value be not required.

If the amounts of barium hydroxide solution used for the titration of the sample and the blank are equivalent to x ml and x_b ml 0·1 N, respectively, the Reichert value, $R = (x - x_b) \times \frac{110}{100}$.

(*P*)—POLENSKE (OR INSOLUBLE VOLATILE ACID) VALUE

Titrate the alcoholic solution of the insoluble volatile acids after addition of 0·25 ml of phenolphthalein solution with 0·1 N barium (or sodium) hydroxide solution, until the solution becomes pink.

If the amounts of barium (or sodium) hydroxide used for the titration of the sample and the blank are equivalent to y and y_b ml 0·1 N, respectively, the Polenske value, $P = (y - y_b)$.

(*K*)—KIRSCHNER VALUE

Add 0·5 g of finely powdered silver sulphate to the neutralised solution from *R*, above. Allow the flask to stand in the dark for one hour, with occasional shaking, and filter the contents through a dry filter. Transfer 100 ml of the filtrate to a dry Polenske flask, add 35 ml of cold distilled water (recently boiled for 15 minutes), 10 ml of dilute sulphuric acid solution, and a loosely-wound 5 mm coil of 30 cm of aluminium wire (about 1 mm thick or S.W.G. about 18 to 20), or 0·1 g of pumice powder. Connect the flask with the standard apparatus and repeat the process as described above, i.e. the distillation of 110 ml in 19 to 21 minutes, the mixing (but without cooling for 10 minutes), the filtration, and the titration of 100 ml of the filtrate with 0·1 N barium hydroxide solution.

If the amounts of barium hydroxide solution used for the titration of the fat and the blank are equivalent to z ml and z_b ml of 0·1 N, respectively, then

$$K\text{, the Kirschner value} = (z - z_b) \times \frac{(100 + a) \times 121}{10{,}000},$$

where *a* represents the actual volume in millilitres of barium hydroxide solution used in the titration for determination of the Reichert value (see above).

Notes—(1) Polenske values, and, to a much slighter extent, Reichert values, have been found to be low when determined at low barometric pressures, such as may occur at high altitudes. The following factors may be applied to values determined at a barometric pressure of p mm of mercury, to convert them to the values determined under normal pressure (Kirkham, 1920)—

Corrected Reichert value

$$= \left(\frac{(\text{observed value} - 10) \log 760}{\log p}\right) + 10$$

Corrected Polenske value

$$= \text{observed value} \times \left(\frac{760 - 45}{p - 45}\right).$$

(2) In order to facilitate weighing the 5 g of the sample, a pipette may be calibrated to deliver 5 g of melted butter fat, any final adjustment to the weight being carried out by the usual means.

(3) Some workers prefer to use a closer textured filter-paper than the No. 4 Whatman for filtering off the insoluble volatile fatty acids; a No. 1 Whatman

filter-paper will be found suitable. In the case of coconut oil, a No. 5 Whatman, and even refiltering, may be necessary to get a clear filtrate.

(4) Genuine butter fat usually gives Reichert values between 24 and 33. In routine examinations of butter fat it is therefore usually sufficient to carry out the Reichert part of the process, and if the figure obtained is above 24 it is unnecessary to carry on with the Polenske and Kirschner. The figure of 24 is only presumptive, as samples of genuine butter have been known to give lower figures (see p. 230).

Rapid method for the determination of small amounts of butter fat in margarine

The following process is a modification, due to Elsdon and Smith (1927), of a method originally suggested by Gilmour (1920).

Saponify 5 g of butter fat with glycerol and sodium hydroxide and distil the volatile acids as in the standard Reichert-Polenske process, with the exception that the distillation is stopped when 100 ml have been collected, in place of the 110 ml as is usual. Add 30 g of pure, dry sodium chloride to the distillate, thereby bringing the volume up to 110 ml. When the whole of the sodium chloride has dissolved, allow the solution to stand for half an hour at 15° C; filter, collect 100 ml of the filtrate, and titrate with 0·1 N sodium hydroxide solution to phenolphthalein. Correct the figure obtained for any acidity found in a blank experiment; the corrected figure, multiplied by 11/10, gives the salt-soluble volatile acid figure.

Determine the salt-insoluble volatile acid figure in exactly the same way as for the Polenske process, except that three quantities of 15 ml of sodium chloride solution (30 g in 100 ml of water) should be used in place of the three quantities of 15 ml of water to wash the condenser, flask and filter-paper.

The method of calculating the percentage of butter fat present is given on p. 263, and from Table 11.12 the approximate percentage may be obtained at a glance.

OTHER CHEMICAL PROCESSES OF BUTTER-FAT EXAMINATION

The A.O.A.C. (1950) describes a process which is a combination of the saponification value, and the determination of soluble acids and insoluble acids. The method is as follows.

Saponification number (Köttstorfer number)

Weigh accurately about 5 g of the sample into a 300-ml flask. Add by pipette 50 ml of alcoholic potash (40 g per litre), reflux for 30 minutes, cool and then titrate with 0·5 N hydrochloric acid to phenolphthalein. Determine a blank experiment at the same time. From the number of millilitres of 0·1 N hydrochloric acid used in the blank, deduct the number of millilitres used in titrating the experiment, the remainder being the number of millilitres of 0·1 N hydrochloric acid equivalent to the potash used in the saponification of the weight of sample taken. Calculate as milligrams of KOH required to saponify 1 g of fat.

Determination of soluble fatty acids

Evaporate the alcohol from the above on a water-bath; then add 0·5 N HCl equivalent to the 50 ml of alcoholic potash used and 1 ml of 0·5 N HCl in excess. Place on steam bath until the fatty acids have completely separated, fill the flask completely with hot water, and cool to solidify the fatty acids. Pour the liquid through a filter-paper into a litre flask. Repeat this treatment of the fatty acids with hot water three times, cooling and collecting the washings into the litre flask each time.

Titrate the contents of the litre flask with 0·1 N sodium hydroxide to phenolphthalein. Subtract 5 (corresponding to the excess of 1 ml of 0·5 N hydrochloric acid) from the number of millilitres used and multiply the remainder by 0·0088, thereby returning the weight of soluble acids as butyric acid. Calculate in terms of percentage on the fat.

Determination of insoluble fatty acids (Hehner value)

The original flask and filter-paper containing the insoluble acids (above) are allowed to drain and dry for 12 hours. The cake and as much as possible of the solid acids on the filter-paper are transferred to a weighed flask. The remaining traces of fatty acids in the first flask and filter-paper are washed into the weighed flask by means of hot alcohol. Distil off the alcohol, dry at 100° C for two hours, cool and weigh. Again heat for two hours and weigh, and repeat if necessary until practically constant. Calculate in terms of percentage of insoluble fatty acids.

The next process deals with a method for detecting the presence of oils of the coconut family by measuring the solubility of the fatty acids in dilute alcohol. Lauric and myristic acids, which are present in large amounts in these oils, are soluble in dilute alcohol.

Modification of the Shrewsbury and Knapp (1910, 1912) method

This combines a modification of the S. & K. method due to Elsdon and Bagshawe (1917) with the Reichert–Polenske–Kirschner process, and is carried out as follows.

The flask containing the residual fatty acids after the distillation of 110 ml in the Reichert–Polenske process is removed from the condenser, and the contents cooled in water until the acids have become a solid cake. The cake is broken and the liquid strained through a fine wire sieve, the flask and fatty acids being washed with 50 ml of cold water. The fatty acids are allowed to drain on the sieve until practically free from water, when they are returned to the flask, the last portions being removed with a thin iron spatula; no difficulty has been experienced in removing the last traces in this way. The flask and contents are then dried in the oven, air being blown through the flask at intervals.

One hundred ml of alcohol (industrial methylated spirit), sp. gr. 0·920 at 15·5° C, are next added from a carefully graduated pipette, the flask corked, and the alcohol heated until the fatty acids have completely dissolved. The alcohol is then cooled below 15·5° C, thoroughly shaken, and allowed to stand in water at 15·5° C for half-an-hour or so until its temperature is exactly 15·5° C. It is then filtered, and 50 ml of the filtrate titrated with 0·1 N caustic soda after the addition of 1 ml of 0·2 per cent phenolphthalein solution. In the case of fats containing large quantities of coconut oil, the end-point is rather vague, and a little practice is necessary before it can be judged exactly. No water should be added.

By this process Elsdon and Bagshawe obtained figures of between 9·0 and 13·0 on fourteen samples of butter. They assume that oils of the coconut group are present if the figure obtained exceeds 14·0.

References to other methods depending on the solubility of fatty acids in diluted alcohol are given on p. 227.

Determination of baryta value—*Avé-Lallemant method*

Avé-Lallemant (1907) modified a method for determining the baryta value of fats which was first described by König and Hart (1891). The modified procedure is as follows.

Two grams of the fat are saponified with excess of alcoholic potassium hydroxide solution, and the solution is titrated back, exactly as in the Köttstorfer process. The neutral soap solution is evaporated to dryness, 10 ml of water being then added, and the evaporation continued so as to remove the alcohol completely. The dry residue is now dissolved in boiling water, and the solution is transferred to a 250-ml flask; the volume of the solution should be about 180 ml. The flask is placed on a boiling water-bath for five minutes, and 50 ml of 0·2 N barium chloride solution are added with constant shaking. After standing on the water-bath for a further 15 minutes, the flask is removed, and when the contents are cold, the volume is diluted to the mark. The mixture is then filtered, the first portion of the filtrate being again passed through the filter; the barium is estimated in 200 ml of clear filtrate. The barium removed from the solution as barium soap is calculated, and the result, expressed in milligrams of barium oxide per 1 gram of fat, represents the " insoluble baryta value " of the fat. The saponification value of the fat is then calculated into barium oxide, and the result, minus the " insoluble baryta value ", gives the " soluble baryta value ".

The figures are returned as milligrams of barium oxide for 1 gram of fat, (*a*) total, (*b*) insoluble, and (*c*) soluble. The difference $b - (200 + c)$ is always negative for pure butters,[1] but the addition of 10 per cent of either coconut oil or beef fat causes this figure to be a positive quantity. The method is not applicable to very rancid butters.

Determination of " seeding-point " of insoluble acids—*Stokoe's method*

Stokoe (1921) worked out a method for determining the relative amounts of both coconut oil and palm-kernel oil in fat mixtures in which both oils may be present, by taking the solidifying point or " seeding-point " of the insoluble volatile fatty acids. The process depends on the well-known fact that the insoluble fatty acids which distil over in the Polenske test for coconut oil are liquid at room temperature, whereas those from palm-kernel oil under the same conditions are solid. The determination is carried out as follows.

In the standard Reichert–Polenske process, after distilling 110 ml the liquid is cooled and the insoluble acids collected on a filter; the condenser tube is rinsed down with 20 ml of warm water (30°–40° C), which is poured over the filter. The filter-paper is washed with warm water (30°–40° C), and as soon as the last drop of water has drained through, several capillary tubes (internal diameter of 1 mm) are filled to the depth of $\frac{1}{2}$ in. with the now melted acids. Two of the tubes are immediately attached, one on either side, by means of a small rubber band, to a thermometer graduated to read $\frac{1}{10}°$, and the thermometer is fitted into a test-tube by means of a cork having a hole through which the thermometer is placed. The tube is supported in a beaker containing ether, the surface of which should be above the level of the acids in the capillary tubes. A rough idea as to whether the bulk of the acids is from palm-kernel or coconut will have been obtained from the appearance of the acids in the original Reichert–Polenske distillate. If the acids are solid at ordinary temperatures it is necessary to warm the ether to about 30° C. A gentle stream of air is forced through the ether by means of a foot-bellows so as to lower the temperature gradually, the rate of cooling being so adjusted that the temperature of the ether is 2° C below that registered by the capillary tube thermometer. As the cooling progresses, the fatty acids will become slightly clouded; then a distinct " seeding " or crystallising will occur. At the first appearance of crystals the temperature is noted. This " seeding-point " is quite definite and sharp. At this point the acids in the capillary tubes appear liquid, with a number of tiny white crystals along the sides of the tube. It is important in performing the test that the rate of cooling should be regular.

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[1] But see p. 242.

Some of the results obtained by Stokoe are given on p. 226.

Determination of the mean combining weight of the insoluble fatty acids

The fatty acids are dissolved in neutral alcohol, a little phenolphthalein added, and titrated with alcoholic alkali; when a pink colour is obtained a small excess is added (2 or 3 ml), the solution heated to boiling for ten minutes, and the excess titrated back, as in the Köttstorfer process.

The mean combining weight of the fatty acids is calculated as the saponification equivalent. Direct titration of the fatty acids does not yield correct results, owing to the formation of anhydrides on drying.

From the difference between the saponification value and the potash used for the insoluble fatty acids, an estimation of the soluble fatty acids can be obtained.

Hawley (1940) has determined the neutralisation value of butter fat as follows. About 10 g of the fat, 150 ml of water, and 30 ml of a solution of 84 g of NaOH in 210 ml of water which has been made to 1 litre with glycerol are saponified in a flask fitted with an air condenser on a water-bath. While still hot, 10 ml of approximately 17 per cent (by volume) sulphuric acid are added; this has been previously adjusted by titrating a blank to methyl-red indicator so that 9·0 to 9·5 ml serve to neutralise the alkali present. After not more than three hours on the water-bath, with frequent shaking, the fatty acids are clarified and 50 ml (which includes most of the fatty acids) are poured off and washed three times with 25 ml portions of hot water in a separating-funnel. The acids are filtered in a steam oven and about 5 g of the filtered acids are accurately weighed into a flask. The acids are dissolved in 50 ml of 90 per cent alcohol (neutralised to thymol blue) and the solution is titrated with freshly prepared 0·5 N caustic soda solution, with 1 ml of 0·04 per cent thymol-blue solution as indicator, until the yellow colour has changed to a slate-green shade. The neutralisation value is the number of milligrams of potassium hydroxide required to neutralise 1 gram of the fatty acid.

Determination of the acid value

Weigh accurately 5 to 10 g of the fat into a 300-ml flask, add 25 to 50 ml of alcohol which has been boiled and neutralised while still warm to phenolphthalein, and heat the contents of the flask on a water-bath until the contents are melted and thoroughly mixed. Titrate the solution with 0·1 N caustic potash or caustic soda to phenolphthalein; shake vigorously when nearing the end-point. Return as milligrams of caustic potash required to neutralise the free acid in 1 g of the fat.

$$\text{Acid value} = \frac{\text{titration} \times 0{\cdot}0056 \times 1{,}000}{\text{grams of fat taken}}$$

Determination of the saponification value

Weigh accurately about 2 g of the fat into a 300-ml flask. Add from a burette 25·0 ml of approximately 0·5 N alcoholic KOH (made by dissolving 35 to 40 g of KOH in 25 ml of water and making up to 1,000 ml with 95 per cent alcohol and, after standing overnight, decanting off the clear liquid). Reflux on the water-bath under a condenser for half an hour and titrate with 0·5 N hydrochloric acid to phenolphthalein. Carry out a blank determination, which should also be refluxed for half an hour. The difference between the blank and the experimental titration represents the KOH used in saponifying the fat. Return as milligrams of KOH required to saponify completely 1 g of the fat.

$$\text{Saponification value} = \frac{(\text{blank} - \text{expt.}) \times 0{\cdot}02805 \times 1{,}000}{\text{grams of fat taken}}$$

It will be noted that the saponification value includes the amount of KOH used to neutralise any free fatty acid present. The difference between the saponification value and the acid value may be termed the " ester value " of the fat.

Determination of the iodine value—*Wijs method*

Reagent. Dissolve 8 g of iodine trichloride in about 250 ml of glacial acetic acid and dissolve 9 g of iodine[1] in about 500 ml of glacial acetic acid. After mixing the two solutions, make to 1 litre with glacial acetic acid, and store in a yellow glass-stoppered bottle.

The amount of fat taken for the test should be such that not more than half the available iodine is absorbed, i.e. the back-titration should not be less than half the blank titration. A suitable amount of genuine butter fat for a determination is 0·4 to 0·5 g.

Method. Weigh accurately a suitable amount of the fat on a small watch-glass or very small glass dish of about 1 ml capacity. Place the watch-glass and contents in a glass-stoppered bottle of about 300 to 500 ml capacity, which has previously been washed and thoroughly dried. Dissolve the fat in 10 ml of dry chloroform or carbon tetrachloride and add accurately from a pipette 20·0 ml of the Wijs reagent. Stopper the bottle and place it in the dark at room temperature for half an hour. Add 15 ml of a 10 per cent solution of potassium iodide and 100 ml of water. Titrate with 0·1 N sodium thiosulphate to starch indicator, shaking vigorously towards the end of the titration. Carry out a blank determination in exactly the same manner. The difference between the two titrations represents the number of millilitres of 0·1 N thiosulphate equivalent to the iodine absorbed by the amount of fat taken. Return as percentage by weight of iodine absorbed by the fat.

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[1] The proportions of iodine trichloride and of iodine must be such that iodine monochloride is formed without an excess of chlorine being present.

$$\text{Iodine value} = \frac{(\text{blank} - \text{expt.}) \times 0{\cdot}01269 \times 100}{\text{grams of fat taken}}$$

Determination of unsaponifiable matter—*S.P.A. method*

Similar methods for carrying out this determination were published in the Report of the S.P.A. (1933b) Sub-Committee on the determination of unsaponifiable matter in oils and fats and in the British Pharmacopoeia. The method is as follows.

Saponification. Weigh accurately a quantity of the oil or fat not exceeding 2·5 g, but not less than 2·0 g, and saponify by boiling for one hour, with occasional swirling, under a reflux condenser with 25 ml of approximately, but not less than, 0·5 N alcoholic potassium hydroxide solution.

Dilution. After the saponification, during which no loss of alcohol should occur, transfer the alcoholic soap solution to a separating-funnel, washing in with 50 ml of water in all.

Extraction. Extract the soap solution, while still just warm, successively three times with 50 ml of ethyl ether. Use the first quantity of ethyl ether to wash out the saponification flask before adding to the soap solution in the separating-funnel.

Make each extraction by shaking the separator vigorously, allowing the two layers to separate and clarify, running off the aqueous alcoholic layer at the bottom of the separating-funnel, and pouring the ethereal solution from the top of the separating-funnel into another separating-funnel containing 20 ml of water. If the ethereal extracts contain solid suspended matter, pass them through a dry, fat-free filter into the second separating-funnel, washing the filter subsequently with ethyl ether.

Preliminary water-washing. Rotate the extracts gently without violent shaking with the 20 ml of water and, after allowing to separate, run off the wash water. Then wash the ethereal solution twice with 20 ml of water, with vigorous shaking on each occasion.

Alkali- and water-washing. After one or other of these preliminary treatments, wash the ethereal solution three times with 20 ml of 0·5 N aqueous potassium hydroxide solution by shaking vigorously on each occasion, each alkali wash being followed by a wash with 20 ml of water. After the last 0·5 N aqueous potassium hydroxide treatment, wash with two or more successive quantities of 20 ml of water until the wash water is no longer alkaline to phenolphthalein solution.

Solvent removal. Transfer the ethereal extract to a weighed flask, distil off the ethyl ether, and dry the residue to constant weight, preferably with the aid of acetone, not allowing the temperature to exceed 80° C.

Acidity test. After attaining constant weight, dissolve the contents of the flask in 10 ml of freshly-boiled and neutralised 95 per cent alcohol, and titrate with 0·1 N alcoholic sodium hydroxide solution, phenolphthalein solution being used as indicator.

Provided that, when the determination is carried out in the above-described manner, the amount of 0·1 N alcoholic sodium hydroxide solution required does not exceed 0·1 ml, take the unsaponifiable matter as being the amount weighed. If the quantity of 0·1 ml of 0·1 N sodium hydroxide is exceeded, repeat the determination from the start, as this limit may correspond with 0·11 per cent of free fatty acid or much larger quantities of acid soap.

Determination of the melting points of the steryl acetates

The melting-point of pure cholesteryl acetate is about 114° C, whilst that of phytosteryl acetate (probably a mixture) is about 124° C. The unsaponifiable matter of animal fats contains cholesterol, whilst the unsaponifiable matter of vegetable oils contains phytosteryl. The determination of the melting-point of the sterol acetate from a sample of fat will show definitely whether it is of purely animal origin or whether vegetable fat is present; therefore the process can be used to detect vegetable oils in butter fat. The process cannot, however, be used to show the presence of animal fats in mixtures containing vegetable oils.

Steuart's (1923) method is as follows. Warm the unsaponifiable matter from 50 g of fat with 50 ml of 95 per cent alcohol and mix with 50 ml of hot 90 per cent alcohol containing 0·5 to 1 g of digitonin. Stand overnight and filter off the precipitate of digitonin steride, wash on the filter with 95 per cent alcohol and with ether, and weigh after drying at 110° C.

$$\text{Percentage of sterol} = (\text{wt} + 0{\cdot}014) \times 0{\cdot}243 \times 2$$

Transfer the steride to a small beaker and add about 1 ml of acetic anhydride for each 0·1 g of steride, cover the beaker with a watch-glass, and boil until the steride is dissolved. Nearly all the steryl acetate crystallises out on cooling. Filter off and dry the crystals with the aid of suction, dissolve through the filter with ether, evaporate the ether, and then dissolve in absolute alcohol. Filter as soon as the crystals separate and obtain several crops of crystals from the alcohol. Place the wet crystals on a porous tile at a temperature of 37° C overnight, and then determine the melting point.

Hawley (1933b) has suggested the following procedure, which has the advantages that the isolation of the unsaponifiable matter is unnecessary and that less sample is required for the determination.

Twenty-five grams of butter fat with 10 ml of chloroform and 15 ml of a 1 per cent solution of digitonin in 95 per cent alcohol are shaken by hand in a small flask for about 10 minutes in a water-bath at 65° to 70° C. The mixture is then filtered hot through a Jena filter (No. 11A, G4). This has a disc of 40 mm diameter, is 90 mm high above the disc, and holds about 100 ml. For this filtration it is essential that the No. 4 (fine grade) be used. The filter is jacketed with water at about 60° to 70° C. A jacket is easily arranged by fixing the filter through a bung in the neck of an inverted, wide, shallow bell-jar which is filled with hot water. The filtrate is turbid, and gives the impression that some of the digitonide has passed through the filter. This will be found not to be the case. The turbidity is due to separation of the fat–chloroform–alcohol mixture on cooling. On adding more chloroform, or warming, the filtrate becomes clear. The digitonide on the filter is washed five or six times with chloroform, each addition being made just before the precipitate is dry, otherwise channels form and thorough washing (which is essential) becomes difficult. Air is now drawn through the filter until the precipitate appears to be dry. The filter is then placed in a vacuum desiccator at two or three mm pressure for half an hour or longer. It will be found that the digitonide has assumed a paper-like texture. The bulk of it is easily removed with a mounted needle.

The pure digitonide is transferred to a long-form Stokes tube, in which it is boiled with 5 ml of acetic anhydride over a very small flame, the neck of the tube acting as an air condenser. Heating is continued for a minute or so after the precipitate has dissolved (about 5 minutes in all). The tube is then rinsed out with 20 ml of 50 per cent alcohol into a small beaker, where the liquid is allowed to crystallise. The crystals are filtered off on a Jena filter; No. 3 (medium grade) is sufficient, and size 3G3 (capacity 30 ml) is convenient. The crystals are washed thoroughly with 50 per cent alcohol and dissolved off the filter with ether, the solution being received in a 10-ml stoppered tube-form weighing-bottle. Ether is removed by warming and blowing in air. The dry steryl acetate is then dissolved by warming in 5 ml of 90 per cent (by volume) alcohol and allowed to crystallise. Absolute alcohol is unsatisfactory with such small quantities of material. The crystals are filtered off through a Jena micro-filter (No. 12G3, capacity 2 ml) and washed four times with 1 ml of cooled 90 per cent alcohol from a pipette. The filter is then dried overnight in a vacuum desiccator, and the melting point determined. In the absence of vegetable fat the melting-point is invariably between 114° and 115° C. By repeated crystallisation it is possible to raise it to about 115·2° C. In the presence of as little as 10 per cent of vegetable oil, the melting-point is over 117° C, and can be raised usually to over 120° C by another crystallisation. Five per cent of vegetable fat can be detected with certainty by two crystallisations.

Determination of thiocyanogen value—*Kaufmann's (1928) reagent*

Add 10 per cent of acetic anhydride to pure glacial acetic acid (99–100 per cent) and allow the mixture to stand at least a week before use. Add 25 g of carefully dried, pure lead thiocyanate to 500 ml of the solvent and add 3 ml of bromine to another 500 ml. Add the latter solution to the former and shake until colourless. Allow to settle and then filter through a dry filter-paper.

Determination. Arup's (1932a) method for the determination on butter fat is as follows. Weigh 0·25 to 0·3 g of the fat into small tubes and place these into 150-ml conical flasks, furnished with well-fitting glass stoppers. Warm the flasks very gently to melt the fat and add 25 ml of the acetic acid and acetic anhydride mixture warmed to 38° C, followed, as soon as the fat is dissolved, by 25 ml of the thiocyanogen solution. Place the absorption flasks and blank experiment in an incubator at 25° to 26° C for 7 hours (for butter fat). Pour the contents of the flasks into 40 ml of 5 per cent potassium iodide solution and then titrate the liberated iodine with 0·1 N sodium thiosulphate to starch indicator; towards the end of the titration rinse out the remains from the absorption flask with some of the partially-titrated solution and continue the titration to the end-point.

The thiocyanogen value is expressed, like the iodine value, in terms of iodine absorbed. The difference between the iodine and thiocyanogen values, divided by the factor 0·905, gives the percentage of linoleic acid.

The Valenta test: solubility of fat in acetic acid

This test, first suggested by Valenta (1884), takes the form of an indirect reading of the solubility of the fat in acetic acid of a definite strength, by taking the temperature at which a turbidity is first noticed when a warm mixture of the fat and acid is allowed to cool. The test has been studied by many workers, but the most complete examination of the method is due to Fryer and Weston (1918). In previous modifications a very slight variation in the strength of the acetic acid made a big difference in the reading obtained; in order to avoid this, Fryer and Weston standardised the acetic acid by means of pure English expressed almond oil of a known degree of acidity; the latter was allowed for by a correction based on the results of experiments on oils of varying acidity. Details are given below of the technique suggested by these workers.

REAGENTS

(1) *Acetic acid.* Glacial acetic acid is allowed to solidify, and the crystals so obtained are drained from adhering liquid at 15° C. They are then melted and the test with almond oil carried out as described below. Should the test give a figure varying from 70° to 90°, the acid is suitable for use, although a figure as near to 80° as possible is desirable. If the figure obtained by the test is above 90°, a further freezing and draining of the crystals is necessary, whilst if below 70° water is added to the acid until a suitable figure is obtained. The amount of water necessary is about 0·029 per cent for each degree rise in temperature required.

The acid is preferably kept in an ordinary vacuum flask. It will be then very unlikely to freeze during the night in cold weather. If this is not available, an ether bottle will suffice, but the acid must be melted when frozen, and will then probably change in value. A 2-ml pipette with a long stem is kept permanently immersed in the acid, being held tightly in a good cork, the opened end kept closed by means of a piece of rubber tube provided with a cap of solid glass rod, the whole forming a close cover. It is thus unnecessary to suck the acetic acid into the pipette for measurement, and accession of moisture from this cause is avoided.

(2) *Standard oil.* This should be English expressed almond oil, which should be kept in a well-corked bottle, and the acidity determined at intervals. The figure obtained is corrected for acidity as described below.

(3) *Preparation of the oil.* The oil must be absolutely free from moisture or the results will be useless. The condition is best brought about by filtering the warm oil through thick filter-paper. Fryer and Weston state that the oil should further be placed in a test-tube immersed in boiling water, and that a thick wad of cotton-wool should then be forced to the bottom of the tube by means of a stout wire plunger; in this way all traces of moisture will be removed.

METHOD. A test-tube is used of such diameter that the bulb of the thermometer is completely covered by 4 ml of liquid. The thermometer is inserted tightly into a cork fitting the test-tube, a small groove being cut in the side of the cork for escape of hot air. A graduation mark is made on the test-tube with a diamond or file at 2 ml from the bottom. Pour the oil into the tube up to the 2 ml mark at the temperature of boiling water (Fryer and Weston have shown that the results are not affected by slight variations in the proportions of the oil and solvent) and measure in also 2 ml of the acid at about 20° by means of the pipette. Insert the thermometer and heat, whilst shaking carefully, over a naked flame until the mixture clears. Then allow to cool slowly, shaking all the time, and take the temperature at which the first signs of turbidity appear. The acid value of the oil is determined, and the experimental figure corrected for this, and also for the reading actually obtained at the same time with the sample of almond oil as described below. (This last precaution is necessary as the acetic acid reagent readily changes in strength on keeping.)

Fryer and Weston carry out the test with two thermometers, one having a small bulb and reading to 0·5°, and the second having a large bulb and reading to 0·1°. They first carry out the test as described above, using the small-bulb thermometer. Then they repeat the determination, using the large-bulb thermometer (graduated to 0·1°), but this time place the tube in water heated to about 5° above the temperature found in the former determination and shake until clear. The tube is then allowed to stand immersed in the hot water until the faintest sign of turbidity appears, when the exact temperature is noted. This refinement will give accurate results suitable for exact work, but will hardly be necessary for routine examinations when, in general, the single determination will suffice.

CALCULATION

(1) *Correction for acidity.* The correction for acidity of the oil should first be made. Ascertain, by reference to the following table, the correction for 1 per cent acidity for the class of oil under test, multiply this by the acidity found, and add the result to the temperature figure obtained.

Table 23.2—Influence of acidity of oils on Valenta figures

Class of oil	Typical oil	Fall in turbidity temp. degrees per 1 per cent acidity (as oleic)
Marine	Whale	1·90
Drying	Linseed	1·85
Semi-drying	Cotton-seed	1·77
Non-drying (except rape and castor)	Almond	2·27
Vegetable fats (except coconut group)	Palm	2·10
Rape-oil group	Rape	2·23
Coconut oil and palm-kernel	Coconut	1·73
Animal fats (except butter fat)	Lard	2·15
Milk fats	Butter fat	1·41

If the class of the oil is unknown, a figure of 2·0 may be allowed in all cases, but the acidity of the oil, for this test, should preferably be low.

(2) *Correction for acetic acid.* The figure given by the acetic acid reagent with the standard almond oil must, where it differs from 80°, be corrected by means of the formula—

$$V = t + (80 - t')$$

where V = true Valenta value;
t = temperature obtained with oil tested, corrected for acidity;
t' = temperature with standard oil and the same acid (correcting for acidity if necessary).

If the standard almond oil is not neutral, the acidity must be ascertained, and the correction made in all cases.

(3) *Results.* Fryer and Weston suggested that the final figure should be returned in the form

$$\frac{V \times 10}{80}$$

thereby showing that all the necessary corrections have been made. By this method they obtained figures approximating to 10 in the case of almond oil, 11 for lard and tallow, 4·7 for butter fat and 1·5 for coconut oil.

The Crismer test

A similar test to the above, depending, however, on the use of alcohol instead of acetic acid, was first suggested by Crismer (1895) and has since been investigated by several workers. The modification due to Fryer and Weston (1918) has, however, completely replaced the methods of earlier workers; this depends on the use of a mixture of equal volumes of amyl alcohol and industrial methylated spirit diluted with water to give a reading of 70° with pure almond oil, after making an allowance for the acidity of the latter.

Determination of hydrogenated oils: percentage of iso-oleic acid in the solid fatty acids

Three methods are available for this determination: the method of Twitchell (1921), the method of Cocks *et al.* (1931) and the lead salt–ether method of Bolton (Williams, 1950). Twitchell's method uses 95 per cent alcohol as the means of separating the lead salts of the liquid and solid fatty acids; this process is probably the one most frequently used. The method of Cocks, Christian and Harding uses 92 to 93 per cent alcohol for the precipitation and petroleum ether for washing the solid lead salts; results by this method are generally appreciably higher than by the Twitchell method. Bolton's method usually gives results for iso-oleic acid which are slightly less than those obtained by the standard Twitchell process.

Twitchell's method

The experimental details given below are practically identical with the original Twitchell process. Weigh into flat-bottomed flasks of 300 ml capacity as much of the mixed fatty acids as is estimated to contain 1 to 1·5 g of solid acids. In the case of very liquid oils this will be about 10 g, while in the case of a tallow it will be only 2 to 3 g. Dissolve in 50 ml of boiling 95 per cent alcohol. Dissolve 1·5 g of lead acetate in 50 ml of boiling 95 per cent alcohol. Heat both solutions to boiling and pour the lead acetate solution into the solution of fatty acids. Allow to cool slowly to room temperature, and then for several hours, preferably overnight, at about 15° C. Filter on a Buchner funnel and test the filtrate for lead with a few drops of an alcoholic solution of sulphuric acid. It there is no precipitate, showing that lead is not in excess, repeat the analysis, using less fatty acids or more lead acetate. Wash the precipitate with 95 per cent alcohol until dilution of the filtrate with water no longer gives an appreciable turbidity (5 washings with alcohol, 20 ml at a time, are usually sufficient). Wash the precipitate back into the container (used in the first process) with about 100 ml of 95 per cent alcohol. The amount of alcohol required is conveniently gauged by marking the height of the original solution in the flask before it is filtered off. It is also convenient to transfer the bulk of the precipitate by means of a spatula and then to wash the Buchner and filter-paper with the alcohol. Boil the mixture under a reflux condenser, with the addition at intervals of a few drops of glacial acetic acid, until the precipitate dissolves. The quantity of acetic acid added should not be greater than 0·5 ml per 100 ml of alcohol. If a greater quantity of acetic acid is needed to effect solution, add a further quantity of 95 per cent alcohol to reduce the amount of acetic acid to this dilution. Continue boiling, etc., until all traces of material (sometimes gum-like) are dissolved. Allow the solution to cool slowly to 15° C and to stand as before.

After standing overnight, filter the solution and wash the precipitate thoroughly as before with 95 per cent alcohol. Transfer the precipitate by washing the filter-paper with ether into the original container. Add sufficient dilute nitric acid to decompose the lead salts. Pour and wash the whole mixture into a separating-funnel and extract the fatty acids with ether. Wash with water until the washings are no longer acid to Congo red. If a trace of nitric acid should remain with the ethereal solution it will act on the fatty acids in the subsequent drying. Filter the ether into a tared flask, distil off the ether, dry, and weigh the solid fatty acids. If desired, the process can be carried out on larger quantities of material than those given above.

From the weight of solid fatty acids and the determination of their iodine value the percentage of solid saturated acids in the fatty acid mixture can be determined. For this purpose, an iodine value of 90·1 is assumed for either oleic acid or iso-oleic acid, and it is further assumed that other unsaturated acids are absent.

By this process, the solid acids of the common oils and fats give iodine values of less than 3, with the exception of tallow, which may give a figure up to 5. Should, therefore, iodine value figures be obtained which exceed the above amounts, there is an indication that iso-oleic acid and, therefore, hydrogenated oils may be present.

The above method usually underestimates the percentage of solid saturated acids, as determined by ester fractionation, by from 1 to 3 per cent in the case of the more common vegetable oils (except the coconut group). If the mixed fatty acids contain an appreciable amount of myristic acid and lower fatty acids (coconut and butter groups), the value of the saturated fatty acid content will be several units below the true figure.

The following table gives typical analyses due to Twitchell by this process—

Table 23.3—Results of lead salt–alcohol process (*Twitchell*)

Oils	Wt. fatty acid (g)	Lead acetate (g)	Alcohol for each precipitate (ml)	Weight solid fatty acids (g)	Per cent	Iodine value of solid fatty acids
Cotton-seed	5	1·5	100	1·1565	23·13	0·72
	20	4	200	4·8680	24·34	0·56
Partly hydrogenated cotton-seed	2·5	1·5	100	1·2655	50·62	42·21
Soya-bean ..	10	1·5	100	1·7010	17·01	0·85
Olive ..	10	1	100	1·0935	10·93	1·75
Peanut ..	20	3·5	300	3·3165	16·58	0·80
Lard ..	3	1·25	100	1·2005	40·02	0·58
Tallow ..	3	1·5	100	1·6085	53·62	4·38

SPECIFIC COLOUR TESTS FOR ADULTERATION

Baudouin's test for sesame oil

This test consists in shaking the melted fat with a solution of cane sugar in hydrochloric acid; a pink colour in the acid layer indicates the presence of sesame oil. Blank determinations should be carried out without using sucrose, to obviate the possible interference of colouring-matters, etc. The test may be carried out as follows. 0·1 gram of finely-powdered sugar is dissolved in 10 ml of hydrochloric acid (sp. gr. 1·20). Two ml of the oil are thoroughly shaken with 1 ml of the reagent and compared with a blank experiment after standing 5 minutes.

Villavecchia modification. Villavecchia and Fabris (1894) modified this by using a solution of 2 g of furfuraldehyde in 100 ml of alcohol to replace the sugar; 10 ml of the melted fat are shaken thoroughly with 10 ml of hydrochloric acid and 0·1 ml of the reagent; a red coloration indicates the presence of sesame oil. This reaction is very delicate, but is not entirely conclusive. Certain colouring-matters, e.g. turmeric and some coal-tar dyes, give a red coloration with hydrochloric acid alone, and in the presence of these sesame oil cannot be detected, as the colour due to the oil would be masked by that yielded by the dye. Furfuraldehyde and hydrochloric acid alone, after some time, yield a reddish colour; hence a slight pinkish tinge gradually appearing must not be taken to indicate sesame oil, especially if it turns black on standing. Spampani and Daddi showed that the milk of goats fed with sesame oil yields butter which gives this test. Hehner, Faber and others were, however, unable to obtain it with butter prepared from the milk of cows fed on sesame cake.

A.O.A.C. modification. The A.O.A.C. have modified the Villavecchia test as follows—

REAGENTS. Hydrochloric acid (sp. gr. 1·19). Villavecchia reagent, 2 ml of furfural added to 100 ml of 95 per cent ethyl alcohol.

PROCEDURE. Mix 10 ml of the sample with an equal volume of the hydrochloric acid. Add to this mixture 0·1 ml of the Villavecchia reagent and shake well for 15 seconds.

Note the colour of the lower layer as soon as possible after the emulsion has broken. If no pink to crimson colour appears, the test may be reported negative at that point. If any colour is observed in the lower layer, add 10 ml of distilled water, shake again, and observe the colour as soon as separation has taken place. If the colour persists, report the test as positive. If the colour disappears, sesame oil is not present.

Arnold (1914) overcame the difficulty of colouring-matters in margarine interfering with the test by the use of stannous chloride. The fat is dissolved in petroleum ether and then extracted with hydrochloric acid containing 0·1 per cent of stannous chloride. The mixture is then heated in a boiling water-bath until the red coloration due to the colouring-matter has disappeared, the furfural reagent is then added, and the test completed in the usual way.

Halphen's test for cotton-seed oil

This test may be carried out as follows. One per cent of sulphur is dissolved in carbon bisulphide and the solution is diluted with an equal volume of amyl alcohol. Equal volumes of this reagent and the sample are placed in a test-tube and heated in an oil-bath at 120°–130° C for one hour. As little as 1 per cent of cotton-seed oil will give a characteristic red coloration under these conditions.

A number of workers, Rupp (1907), Shelly (1925) and others have suggested carrying out the test in a closed tube. In this modification a strong test-tube containing the test mixture is stoppered with a cork or glass stopper which is securely tied down; the tube is then immersed to half its length in a bath of boiling water for at least half an hour.

Gastaldi's (1912) modification of the test was as follows.

Five millilitres of the fat are mixed in a strong test-tube with 1 drop of pyridine and 4 ml of carbon bisulphide containing 1 per cent of sulphur, the tube is closely stoppered and heated in the water-bath for half an hour. A red colour indicates the presence of cotton-seed oil.

Although all samples of cotton-seed oil do not give the reaction to the same extent, nevertheless, in a sample of oil the depth of colour obtained is proportional to the amount of the particular cotton-seed oil present. The test can, therefore, in a very limited way, be made roughly quantitative by making comparative tests with known amounts of cotton-seed oil.

The Halphen test is subject to the following limitations.

Cotton-seed oil which has been heated to above 210° C, or oil which has been hardened or chemically treated in other ways, gives the test with greatly diminished intensity; in fact, if the oil has been heated above 250° C the test fails completely.

Dunlop (1906) and other workers found that the fat of pigs and other animals fed on cotton-seed meal frequently gives a positive reaction in the test. In the case of animal fats, therefore, actual adulteration must not be assumed unless the amount of cotton-seed oil indicated exceeds something of the order of 10 per cent.

The reaction is given by certain other vegetable oils, including kapok oil (Sprinkmeyer and Diedrichs, 1913), calumpang seed oil (Bolton and Jesson, 1915), and the oil of okra seed (Jamieson and Baughman, 1920).

Detection and determination of acetyl-methyl-carbinol and diacetyl

The flavour of butter is due to very small amounts of diacetyl, CH_3–CO–CO–CH_3, which is an oxidation product of the carbinol. The formation of the ketone is a reversible reaction, and it tends to occur in butters of high acid value which have been exposed to the influence of air. On the other hand, diacetyl may disappear altogether in butter which has become tallowy and which has absorbed all the available oxygen. It is therefore somewhat difficult to decide when diacetyl has been added artificially, but its presence in large amounts in butters of low acid value is unusual. Davies (1933b) has given the amounts of diacetyl in fresh butter prepared from ripened cream as being between 0·05 to 0·50 parts per million, and he suggested the following tests for its detection and determination.

Voges-Proskauer reaction. Diacetyl gives a red coloration in strongly alkaline solutions in the presence of peptone solution, creatine, or other substances containing a guanidine nucleus. When it is desired to demonstrate the presence of the carbinol in cultures containing peptone it is only necessary to shake with air to oxidise the carbinol to diacetyl, and then add alkali, when the colour develops. The amount of caustic soda added should be sufficient to give a concentration of 5 per cent in the mixture. The colour fades and the test cannot be used for quantitative purposes, but it is extremely delicate and, as a spot test, will detect the presence of one hundredth of a milligram of diacetyl; it should be used in all cases where the quantitative tests give negative results.

Gravimetric determination. Treat 500 to 1,000 g of butter with a mixture of 500 ml of 0·1 N sulphuric acid and 1 per cent acetic acid, adding a drop of oleic acid to oxidise any carbinol to diacetyl. Reflux for 20 minutes in an all-glass apparatus and then distil 40 ml. Add 10 ml of a buffered nickel reagent (1 part 20 per cent hydroxylamine hydrochloride, 1 part 10 per cent nickel sulphate solution, and 2 parts 20 per cent sodium acetate solution) to the 40 ml of distillate and maintain at 90° C for 1 hour. Cool, neutralise to pH 7·2, and allow to settle for 24 hours. Filter the nickel compound off on a tared, sintered Jena crucible (IG_{33}), dry at 110° C and weigh. The weight of precipitate multiplied by 0·596 equals total acetyl-methyl-carbinol and diacetyl in terms of diacetyl.

Colorimetric method. Distil 100 g of butter and 100 ml of water without the addition of acid and collect 100 ml of distillate in a receiver cooled in ice water. Prepare the nickel compound as directed in the gravimetric method, the 100 ml of distillate being evaporated to a small bulk and then heated for a further three hours under a reflux condenser. Do not neutralise, but collect the precipitate on a filter disc in a Gooch crucible and compare the colour with discs obtained from a series of determinations using known amounts of diacetyl. It has been suggested that a few drops of ferric chloride may be added to the distillation mixture to oxidise any carbinol to diacetyl.

For the most recent work Williams' revision of Bolton's *Oils, fats and fatty foods* should be consulted.

CHAPTER 24

THE ANALYSIS OF CHEESE

A complete analysis of cheese includes determinations of the water, fat, ash, salt, proteins, primary products of ripening (as albumoses and peptones), secondary products of ripening (such as amino-compounds, ammonia, and nitrates), and lactic and fatty acids; also, when present, milk-sugar. Few, however, of these determinations can be made with accuracy, though results which are of great utility can be obtained readily. In addition to the determinations mentioned, the fat may be examined as to its genuineness, and the proteins as to their digestibility.

Sampling

This may be done either by a borer or by cutting a wedge-shaped piece; in either case the sample should be taken in such a way as to include a representative portion of the central and all other portions of the cheese. The rind is then cut off and the remainder of the sample transferred to a sample jar. Hard cheeses may be prepared for analysis in a number of ways, including repeated passage through a mincing machine, grating, or simply cutting into very small pieces about 1 mm cube and then thoroughly mixing. Soft cheeses must be thoroughly mixed with a pestle and mortar, or with the aid of a spatula. In all cases the prepared sample should be kept in an airtight sample jar and exposed as little as possible to the air during the weighing-out of portions for analysis, in order to prevent changes in the water content of the sample, which very readily occur with this commodity.

Methods for the determination of the various constituents of cheese are found in the following paragraphs. Reference should also be made to the processes given in B.S. No. 770—1952 and *Methods of Analysis of the A.O.A.C.*

Determination of moisture

This may be conveniently determined by weighing out about 2 g of the prepared sample, spread in a thin layer in a porcelain or silica milk-dish, or in a platinum, aluminium or German silver, etc., dish, preferably fitted with a lid. The dish is then heated for 3 hours in contact with the metal shelf of an oven maintained at 100° C. If the dish is provided with a cover this is then replaced, the dish allowed to cool in a desiccator and weighed. It is then replaced in the oven and heated until constant in weight for further periods of one hour.

Alternatively, the sample may be weighed into a dish containing clean ignited sand and a glass rod. The cheese is then intimately mixed with the sand before heating in the oven; less time is usually required for the contents of the dish to become constant in weight by this method.

Both of the above processes can be expedited by carrying out the heating under reduced pressure.

Devarda's method

Devarda recommended for the determination of water that about 10 g of finely-divided cheese should be dried *in vacuo* over sulphuric acid for twenty-four to thirty-six hours, and then for two to six hours at 100° C until the weight becomes constant. In this way the bulk of water is removed at the ordinary temperature, and, whilst the method is fairly quick, there is no material loss of organic matter, such as occurs with long-continued drying at 100° C. Complete drying *in vacuo* is too tedious and often impracticable.

The following examples show the accuracy of this process—

Table 24.1—Moisture in cheese: comparison of methods

Name of cheese	Loss of water per cent				Water per cent	
	24 hours *in vacuo*	A second 24 hours *in vacuo*	3 to 6 hours at 100°	Total	Dried at 100° C	Dried *in vacuo*
Romadur ..	46·24	2·24	3·11	51·59	51·92	51·50
Limburger ..	37·79	1·12	0·09	39·00	39·38	38·98
Gervais ..	47·89	—	1·36	49·25	49·36	49·10
Limburger (air-dried) ..	11·10	—	2·17	13·27	13·46	—

Ash

This may be determined on the residue from the moisture determination; or preferably 5 g should be partially dried in the oven and then ignited carefully at a low temperature as for milk ash. Data and methods for calcium and phosphorus are given by E. C. V. Mattick (1951).

Salt

This may be determined on the carefully ignited ash by extracting with water, filtering and titrating with standard 0·1 N silver nitrate, using potassium chromate as indicator. Alternatively, the ash can be dissolved in dilute nitric acid and the chlorides determined by the Volhard process.

The above methods suffer from the objection that the sample must first be ashed, with probability of loss of chlorides due to volatilisation. The following method due to Davies (1932b) avoids this.

Two grams of the sample are weighed into a 250-ml conical flask and well wetted or soaked with water, warming if necessary, and 25 ml of 0·05 N silver nitrate are added. The contents of the flask are thoroughly shaken, 10 ml of saturated potassium permanganate solution and 25 ml of concentrated nitric acid added, and the contents of the flask are boiled over a gauze. If the amount of salt is considerable, or if there is a large volume of unattacked fibrous material, fat, or sand, the cooled digest, after dilution to 100 ml, is filtered by suction, the precipitate being washed repeatedly with hot 5 per cent nitric acid. When, however, the amount of solid material is small, the excess of silver nitrate may be determined at once. The filtrate is made up to standard volume (200 ml) and the excess of silver nitrate is determined in aliquot portions (or on the whole liquid) by titrating with 0·05 N thiocyanate solution, using 1 ml of saturated iron alum as indicator, after adding acetone until the solution contains 5 per cent. If it is found that all the silver nitrate has been used up, the experiment must be repeated on a smaller amount of sample; addition of excess silver nitrate after the acid digestion is completed is useless.

Determination of fat

For routine purposes, this may be determined by the Gerber or Leffmann-Beam process, using the technique described for cheese under the general description of the above processes. For exact work it is necessary to use a gravimetric process. The Röse-Gottlieb being an alkaline process is liable to give low results in that it will not include any free fatty acids present. Modifications of the Werner-Schmid or the Schmid-Bondzynski processes, which rely on acid digestion of the curd followed by solvent extraction of the fat, are suitable. The following method is a slight modification of the one recommended by the Milk Products Sub-Committee to the Analytical Methods Committee of the S.P.A. (1936a) for the determination of fat in dried milk.

Weigh about 1 g of the sample into a hard glass boiling-tube (8 by 1 in.), add 15 ml of hydrochloric acid (sp. gr. 1·125) and boil gently until all particles of curd are dissolved. Cool; add 10 ml of 95 per cent alcohol and mix well. Add 25 ml of ether; close the tube with a moisture-moistened glass stopper or cork (rubber bungs are not suitable); shake well for 15 seconds. Cool, remove the stopper; wash the stopper and the neck of the tube with petroleum spirit and add, including the amount in the washings, 25 ml of petroleum spirit. Replace the re-moistened stopper, shake vigorously for 30 sec., and either allow the tube to stand or whirl in a centrifuge until the two layers of liquid are completely separated.

Transfer the ethereal layer as completely as possible to a suitable flask by means of a siphon or wash-bottle tubes. Wash the tip of the siphon-tube into the flask with ether, disconnect the siphon fitting and wash down the inside of the extraction-tube with 5 ml of ether; without further shaking, siphon off this ether, and wash the tip of the fitting as before.

Add 15 ml of ether to the extraction-tube, using this ether to wash the cork and inner limb of the siphon-fitting before its removal. Replace the freshly-moistened stopper; shake for 15 sec.; add 15 ml of petroleum spirit and shake for 15 sec., taking the same precautions as to washing the neck and stopper as before. When the ethereal layer has separated, transfer it to the flask as before.

Repeat the extraction with 15 ml of ether and 15 ml of petroleum spirit, and the transference of the ethereal layer to the flask as in the last paragraph, and wash the tip of the siphon-tube.

Cautiously distil the solvents from the flask, and then dry the residual fat at 102° to 103° C for 1 hour, removing all solvent vapour from the flask at the early stages of the drying by blowing air gently into the flask. Cool and weigh. Repeat the heating until there is no loss in weight.

Completely extract the fat from the flask by repeated washings with petroleum spirit, allowing any sediment to settle before each decantation, and washing off any fat which may have crept over the edges of the flask during the removal of the fatty solutions. Dry the flask at 102° to 103° C, with removal of solvent vapour, cooling and weighing as before.

The difference in weight before and after the petroleum spirit extraction is the weight of fat contained in the quantity of cheese taken for analysis, uncorrected for the blank.

Make a blank determination, using the specified quantities of reagents and distilled water, and deduct the weight found, if any, from the weight of fat obtained.

Calculate the percentage of fat in the sample as analysed, and also the percentage in the moisture-free sample.

Should the examination of the fat be considered necessary, a portion of the sample is cut up and heated in a porcelain dish in the 100° C oven. If sufficient fat cannot be poured off by this method, the mass is ground up with clean sand and treated with ether, the ether solution filtered and evaporated, and the fat examined by the methods described for butter fat.

Acidity

The A.O.A.C. recommends treating 10 g of the finely-divided cheese with water at 40° C. The mixture is vigorously shaken, the volume adjusted to 105 ml and filtered. Twenty-five ml (2·5 g sample) of the filtrate are titrated to phenolphthalein using 0·1 N caustic soda solution. The results are expressed as per cent of lactic acid (1 ml of 0·1 N caustic soda solution $\equiv$ 0·0090 g lactic acid).

Total protein

One to two grams of the sample are examined by the Kjeldahl process. The percentage of nitrogen multiplied by 6·38 is taken as the percentage of protein. This is not strictly accurate, as part of the nitrogen is present as ammonia and amino-acids, and the true protein is therefore overestimated.

Citric acid

This may be determined by the method of Lampitt and Rooke previously described for citric acid in milk. Twenty-five grams of the finely-divided cheese are vigorously shaken with 100 ml of water at 50° C and the process then carried out as for milk.

Tartaric acid

The A.O.A.C. recommends the following method for detection of tartaric acid. Five grams of the finely-divided cheese are treated with 40 ml of water at 50° C. Three ml of a 1 per cent sulphuric acid solution are added and the mixture vigorously shaken; 2 ml of a 20 per cent solution of phosphotungstic acid are then added and the shaking repeated. After standing for 5 minutes the mixture is filtered. Twenty-five ml. of the filtrate are rendered alkaline with saturated $Ba(OH)_2$ solution and 25 ml of 95 per cent alcohol added. After shaking vigorously the deposit is allowed to settle and is filtered through a Büchner funnel and washed on the filter several times with water. A portion of the deposit is transferred to a small dish and dried on the water-bath. A few millilitres of concentrated sulphuric acid and a few crystals of resorcin are added and the mixture heated slowly. In the presence of tartaric acid a rose-red colour is produced, which is slowly discharged on dilution with water.

Tin

Wrapped and processed cheese may be examined for this metal as follows. Twenty-five grams of the cheese are treated with 12 to 24 drops of concentrated sulphuric acid in a porcelain dish, dried on the hot-plate, carefully charred, and allowed to cool. The contents of the dish are then ground up with 20 g of potassium sulphate and transferred to a Kjeldahl flask. Thirty-five ml of concentrated sulphuric acid are placed in the dish, heated to fuming on a gauze, and also transferred to the Kjeldahl flask. The contents of the flask are then digested until clear, allowed to cool, diluted first with cold water and then with hot water, filtered while hot, the filter washed and the bulk of the filtrate made to about 500 ml. Sulphuretted hydrogen is passed into the solution for 30 minutes and the precipitate is allowed to curdle on the water-bath, preferably overnight. If, after filtering and washing with sulphuretted-hydrogen water, appreciable quantities of tin appear to be present, the precipitate is redissolved on the filter with the smallest possible amount of boiling 2·5 per cent caustic soda solution; the filter is washed with distilled water. Tin is re-precipitated in the filtrate by making slightly acid with acetic acid and passing sulphuretted hydrogen for 20 minutes. After coagulation, the precipitate is filtered, washed with sulphuretted-hydrogen water, dried, ignited and weighed as SnO_2. Then

$$SnO_2 \times 0{\cdot}788 = \text{tin}$$

The amount of tin found should not exceed at the most 2 grains to the pound (286 parts per million).

Determination of proteins and products of ripening—Richmond's method

About 10 grams of cheese are intimately ground in a mortar with ten successive portions of 20 ml each of hot water, the aqueous portions being poured off into a 250-ml flask. The grinding should be as thorough as possible, every lump of cheese being carefully crushed. After cooling, the solution should be made up to 250 ml and filtered.

Twenty-five ml of the filtrate should be evaporated in a platinum basin on the water-bath, and the residue dried in the water-oven to constant weight. This may be termed the " total soluble extract ". The residue may be incinerated at a low red heat, and the ash of the soluble extract weighed.

Twenty-five ml of the filtrate are diluted to about 100 ml, 5 ml of a solution of copper sulphate added (34·64 g to 500 ml), and caustic soda solution is then added, drop by drop, till the precipitate settles in the form of curd, and leaves the supernatant liquid quite clear. After standing for some time, the precipitate is collected on a Gooch crucible, washed with water, and dried at 120° C. After weighing, the crucible is ignited, and the residue of copper oxide and phosphate weighed. The difference between the two weights may be taken as " primary products of ripening ". The difference between this figure and that of the " total soluble extract ", less the ash, may be taken as " secondary products of ripening ". The difference between the " total soluble extract ", less ash of soluble extract, and the solids-not-fat, less total ash, may be taken as proteins.

The above method will be found to be fairly rapid and to give an insight into the composition of the protein matter of the cheese. The separations between the different classes of protein substances are, however, arbitrary. Thus it is assumed that all the insoluble solids-not-fat consist of protein, and that all the products of ripening (and nothing else) are soluble. The distinction between primary and secondary products of ripening is based on the assumption that primary products are precipitated as basic copper compounds, while secondary products give soluble compounds under the conditions given above. In the present state of knowledge it is impossible to identify and separate all the products of ripening; therefore empirical methods which yield comparative results are necessary.

Stutzer's methods

If it be desired to obtain further information, the method given above may be elaborated by some of the methods detailed below. Stutzer has published a study of the methods of cheese analysis, of which the following is an abstract.

Ash and mineral matter. From 10 to 15 grams of the cheese are burnt (preferably in a muffle) in a platinum basin. The weighed ash is dissolved in 250 ml of water and an aliquot portion used for the determination of chlorine (calculated to sodium chloride). The portion insoluble in water may also be dissolved in dilute hydrochloric acid and made up to 250 ml; in a mixture of equal aliquot portions of each of these solutions the calcium and phosphoric acid may be determined.

Water. A weighed quantity of the cheese is mixed with washed, ignited, and sifted quartz sand. For most cheeses the proportion of 100 g to 400 g of sand is satisfactory, but with very rich cheeses 500 g of sand are taken. This sand mixture is used in all the estimations. For the determination of the water, an amount of the mixture corresponding to about 3 g of cheese is dried to constant weight in the water-oven.

Fat. The dry residue from the water-determination is extracted for 24 hours with water-free ether, which has been dried over sodium.

Nitrogen—(1) *Total nitrogen.* Ten grams of the sand mixture are analysed by Kjeldahl's method.

(2) *Applicability of copper hydrate to the precipitation of proteins, etc.* Formerly, Stutzer employed copper hydrate to separate proteins and their primary cleavage products from secondary products (amino-compounds, etc.). He later found that it only precipitates trypto-peptones (pancreas-peptone) partially, and, extending his experiments to cheese, he found that there is sometimes a peptone present which is not completely precipitated.

(3) *Phosphotungstic acid as a precipitant.* The conclusions of Bondzynski that phosphotungstic acid is a suitable separating agent are confirmed. By its means the proteins and their primary cleavage products (albumoses and peptones) are separated from the secondary products (phenyl-amino-propionic acid, leucine, tyrosine, and other amino-compounds) and ammoniacal compounds, all of which Stutzer classed as worthless. The substances belonging to the first group may be divided further into (*a*) indigestible nitrogenous

matters; (*b*) albumoses and peptones soluble in boiling water; and (*c*) proteins insoluble in boiling water.

(4) *Nitrogen in the form of ammoniacal salts.* An amount of the sand mixture corresponding to 5 g of cheese is mixed with 200 ml of water and the ammonia distilled, after the addition of barium carbonate. Magnesia and magnesium carbonate cause a partial decomposition of the amides. Richmond preferred to operate on a portion of the hot-water extract.

(5) *Nitrogen in the form of amino-acids.* This is taken to be the nitrogen belonging to those compounds in the cheese which are not precipitated by phosphotungstic acid, and which are not ammoniacal compounds. An amount of the sand mixture corresponding to 5 g of cheese is mixed with 150 ml of water, and shaken well for fifteen minutes in a closed vessel. After standing for fifteen hours at the ordinary temperature, 100 ml of dilute sulphuric acid (1 vol. to 3 vol. water) are added, and phosphotungstic acid so long as a precipitate results. The liquid is filtered, the precipitate washed with dilute sulphuric acid until the filtrate amounts to 500 ml, and the nitrogen is determined in 200 ml of this. By deducting from the amount previously found as ammoniacal nitrogen, the nitrogen present in the form of amino-acids is found.

(6) *Indigestible nitrogenous substances.* The fresh mucous membrane of six pigs' stomachs is cut into small fragments and mixed with water and hydrochloric acid in a wide-necked flask in the proportion of 5 litres of water and 100 ml of 10 per cent (by weight) hydrochloric acid to each stomach. At the same time, 2½ g of thymol dissolved in alcohol are added as a preservative. The mixture is left for twenty-four hours, with occasional shaking, and then filtered through flannel, coarse paper, and fine paper successively. If necessary, the amount of hydrochloric acid in the extract is brought to exactly 0·2 per cent. As thus prepared, the gastric juice remains unaltered for months.

Sand mixture, containing 5 g of cheese, is deprived of its fat by extraction with ether, mixed with 500 ml of gastric juice in a beaker, and the mixture warmed for forty-eight hours in a thermostat at 37° to 40° C. At intervals of about two hours, 5 ml of 10 per cent hydrochloric acid are added, until the acidity of the whole reaches 1 per cent. The liquid is filtered through paper or asbestos, the residue washed with water, and the nitrogen in it determined.

(7) *Nitrogen in the form of albumoses and peptones.* A weighed quantity of the sand mixture, containing 5 g of cheese, is extracted by boiling with successive portions (100 ml) of water, the liquid made up to 500 ml and filtered; and 200 ml of the clear filtrate (mixed with an equal volume of dilute sulphuric acid) are precipitated by phosphotungstic acid. The precipitate is collected on a filter, washed, and the nitrogen determined by Kjeldahl's method.

Qualitative test for peptones. A portion of the hot-water extract is concentrated by evaporation, saturated with zinc sulphate, and filtered. Concentrated sodium hydroxide solution is added to the filtrate until the zinc hydroxide dissolves, and a few drops of a 1 per cent solution of copper sulphate are added; a violet-red colour (the biuret reaction) points to the presence of peptone. If desired, this may be estimated by evaporating 200 ml of the filtrate to 50 ml, saturating with zinc sulphate, filtering, and washing with saturated zinc sulphate solution; the precipitate, which consists of albumoses, is treated by Kjeldahl's method. The nitrogen in the peptone is the difference between that in the albumoses and that in the precipitate formed by phosphotungstic acid.

(8) *Proteins.* The nitrogen present in these substances, which are insoluble

in boiling water, is obtained by subtracting from the total nitrogen the amounts found in (4), (5), (6) and (7). It is not advisable to use the residue from (7) for this purpose, on account of the large amount of sand present.

(9) *Separation of the proteins digestible with difficulty from those readily digestible.* Cheese contains only small quantities of completely indigestible nitrogenous substances, and it is, therefore, useful to determine the comparative digestibility of the proteins. For this purpose, a process of "interrupted digestion" is employed. In order to obtain comparable results, care is taken to have constant (*a*) the amount of nitrogen in the form of insoluble but digestible proteins; (*b*) the amount of gastric juice; and (*c*) the acidity of the liquid, the temperature, and duration of digestion.

In each experiment so much of the sand mixture is taken as contains 0·15 g of nitrogen in the form of insoluble but digestible proteins, to which is added 150 ml of the gastric juice, with 343 ml of water and 7 ml of 10 per cent hydrochloric acid. The acidity of the total liquid ($\frac{1}{2}$ litre) is exactly 0·20 per cent, the temperature is maintained at 37° to 40° C, and the duration of the digestion is thirty to sixty minutes. The liquids are warmed to 40° C before being measured and after mixing. At intervals of five minutes during the digestion the liquid is stirred with a glass rod; and at the conclusion the total liquid is placed in two large folded rapid filters, and the portion of the filtrate passing through in the first five minutes taken for the determination of the nitrogen. From the result a deduction must be made for the nitrogen contained in the gastric juice, and for the nitrogen in the cheese dissolving without the aid of the gastric juice (amino, ammoniacal, albumose, and peptone nitrogen).

Table 24.2 gives the results of the analyses of three varieties of cheese to illustrate the results of Stutzer's investigation. The nitrogen in the proteins multiplied by 6·38 will give, with fair accuracy, the amount of the proteins; the nitrogen of the albumoses and peptone multiplied by 6·38 will approximate nearly to the amount of primary products of ripening. Richmond has calculated from the nitrogen given in Stutzer's analysis the proteins, primary and secondary products of ripening, in order to compare the method given above with that previously described.

For most practical purposes, Richmond's method will give as much information as that of Stutzer if the following facts are borne in mind: (1) the ripening of a cheese is shown by the proportion of primary and especially secondary products; and (2) the digestibility of a cheese increases with its ripeness.

Van Slyke's method for the estimation of the products of ripening

Twenty-five grams of cheese are intimately mixed in a mortar with an equal volume of quartz sand, and transferred to a flask; 100 ml of water at about 50° C are added, and the mixture kept at 50° to 55° for half an hour with frequent shaking. The liquid is filtered through cotton wool into a 500-ml flask, and the residue is treated as before with four further successive quantities of 100 ml of water; after cooling, the volume is made up to the mark.

Water-soluble nitrogen. This is estimated by the Kjeldahl method in 50 ml of the solution.

Nitrogen as paranuclein. Five ml of a 1 per cent solution of hydrochloric acid are added to 100 ml of solution, and digested at 50° to 55° C till the

Table 24.2—Analyses of cheese (*Stutzer*)

	Camembert	Swiss	Gervais
	Per cent	Per cent	Per cent
Water	50·90	33·01	44·84
Fat	27·30	30·28	36·73
Organic solids-not-fat	18·66	31·41	15·48
Ash	3·14	5·30	2·95
The ash contained—			
Calcium	0·03	1·56	0·14
Phosphoric acid	0·76	0·82	0·23
Sodium chloride	2·21	1·56	0·76
Total nitrogen	2·900	5·072	1·923
Nitrogen as ammonia	0·386	0·188	0·031
,, amino-acids	1·117	0·459	0·099
,, albumose and peptone	0·885	0·435	0·298
,, indigestible matter	0·115	0·119	0·166
,, digestible proteins	0·397	3·871	1·329
Percentage of proteins dissolved by gastric juice in 30 minutes	100	68	52
Percentage of proteins dissolved by gastric juice in 60 minutes	100	91	75
100 parts of nitrogen were present in the following forms—			
As ammonia	13·0	3·7	1·6
,, amino-acids	38·5	9·0	5·2
,, albumose and peptone	30·5	8·6	15·5
,, indigestible matter	4·0	2·4	8·6
,, digestible proteins	14·0	76·3	69·1
The above analyses expressed according to Richmond's method give—			
Water	50·90	33·01	44·84
Fat	27·30	30·28	36·73
Ash	3·14	5·30	2·95
Proteins	3·27	25·46	12·73
Primary products of ripening	5·64	2·77	1·91
Secondary ,, ,,	9·75	3·17	0·84

precipitate has separated completely. The precipitate is filtered and washed, and the nitrogen determined by the Kjeldahl method.

Nitrogen as coagulable protein. Neutralise the preceding filtrate, heat to boiling, and determine the nitrogen in the precipitate, if any; coagulable proteins rarely occur in cheese.

Nitrogen as caseoses. To the filtrate from the last determination add 1 ml of 50 per cent sulphuric acid, saturate with zinc sulphate, and warm to 70° C.

Cool the solution, filter, wash with a saturated solution of zinc sulphate, and determine the nitrogen in the precipitate.

Nitrogen as amino-acids and ammonia. To 100 ml of the aqueous solution of the cheese add 1 g of sodium chloride, and tannin solution till the precipitation is complete; filter and dilute the filtrate to 250 ml, and carry out a Kjeldahl determination on an aliquot portion. This will give the nitrogen as amino-acids and ammonia; the ammonia is determined in another aliquot portion by making alkaline with a little magnesia, and distilling the ammonia into standard acid.

Nitrogen as peptone. The difference between the water-soluble nitrogen and the sum of the other determinations will give the nitrogen as peptone.

Nitrogen as mono-calcium caseinate. The portion of the cheese insoluble in water is treated with successive portions of 100 ml of a 5 per cent sodium chloride solution, in a manner similar to the treatment with water, and the nitrogen as mono-calcium caseinate is determined by the Kjeldahl method in an aliquot portion of the solution.

Other methods are described in the following references: Davies *et al.* 1934, Allen 1930.

Duclaux's method

Duclaux proposed the investigation of the fatty acids developed by ripening as a means of judging a cheese. The following are the methods used by him.

Water, fat, alcoholic and aqueous extracts. Twenty grams of sand which have been previously dried, sifted, and ignited are weighed out, and about seven-eighths are placed in an enamelled mortar; 2 to 3 g of cheese, accurately weighed, are ground up with the sand to form a homogeneous mass, which should become nearly pulverulent. The mixture is introduced into a small calcium chloride tube, fitted with a plug of asbestos to prevent loss, and the basin rinsed out with the remainder of the sand. The tube with its contents are weighed, and placed in a bath heated to 50° or 60°, and a current of dry air passed through for some hours. After cooling, the tube is weighed and the loss noted as water.

The fat is now extracted by carbon disulphide (other solvents, such as ether or chloroform, may be used), the tube again dried and weighed, and the amount of fat deduced by difference.

The tube may be similarly exhausted by alcohol, hot or cold water, and the loss of weight noted after each extraction.

Ash and salt. A fresh portion of cheese is weighed out into a platinum basin, and ignited to obtain the ash; in this, the chlorine is titrated with standard silver nitrate, using potassium chromate as indicator.

Proteins and products of ripening. About 10 g of cheese are weighed and mixed intimately in a mortar with about 10 ml of water; a very homogeneous paste is formed, and this is left for half-an-hour to ensure the perfect contact of the water with the solid matter. More water is added, little by little, the mixing in the mortar being continued till 100 ml have been added. The mixture is now filtered through a porous porcelain filter by means of reduced pressure; in several hours 60 to 70 ml can be obtained.

Ten millilitres are evaporated in a platinum basin, the residue dried at 100°, weighed, then ignited, and the ash weighed. The difference will give the organic matter; this is termed by Duclaux "caseone", and represents the products of ripening. The percentage may be calculated with approximate accuracy, by multiplying by 100 + the weight of water in the amount of cheese taken, and dividing by one-tenth of the weight of the cheese.

The remainder of the filtered liquid (50 ml) is brought, by the addition of water, to 150 ml, and distilled into standard acid to determine the free ammonia; this determination is not very exact, as ammonia is gradually liberated as the distillation proceeds; hence it is usual to stop the distillation when 75 ml have distilled over. A little calcined magnesia suspended in 25 ml of water is next added, and about 50 ml are distilled into standard acid for the estimation of combined ammonia.

The residue in the distillation flask is rendered acid by the addition of a little sulphuric acid, and made up to 55 ml; 40 ml are distilled off, and the volatile acid received in standard alkali. The acid is calculated as butyric by multiplying the number of millilitres of 0·1 N alkali used by the factor 0·00975 (this factor assumes that 90·2 per cent of the total acid will be obtained under these conditions).

Duclaux gives the following analyses—

Table 24.3—Analyses of ripened cheeses (*Duclaux*)

	Curd two days old	Cantal cheese in good condition	Old Cantal cheese
	Per cent	Per cent	Per cent
Water	40·7	44·4	36·26
Fat	30·1	23·9	34·70
Proteins (insoluble)	20·0	13·7	11·09
,, (soluble but not filtrable) ..	4·1	8·3	
,, (filtrable)	4·3	7·2	13·50
Salt	0·8	2·5	2·23
Ammonia, total	—	—	0·90
Volatile acids (as butyric)	—	—	0·27

Volatile acids. The following proportions of volatile acids per 100 of fat are instructive—

Table 24.4

	Per cent
Fresh curd	0·04
Curd five days old	0·55
,, eight ,, ...	2·33
Cheese from the same curd two months old ..	3·0
Cantal cheese	3·2
Fat from above rancid after one month ..	9·2
Salers cheese (bitter)	8·8
,, (taste good)	2·0
Cheese five years old	71·2

Methods for estimating volatile acids in cheese have also been described by Hiscox *et al.* (1941, 1951).

Ramsey and Patterson (1945) have separated and identified the C_1 to C_4 acids by chromatography using a silica gel, and Musicant and Kaszuba (1939) have made use of the well-defined and characteristic crystals of mercurous propionate to analyse a mixture of acetic, propionic and butyric acids.

CHAPTER 25

THE DETERMINATION OF METALS IN DAIRY PRODUCTS

Determination of lead

This metal is to some extent volatile on ignition, and the determination of small amounts colorimetrically as lead sulphide is rendered difficult by the interference of other metals, phosphates, etc. In recent years several methods have been published with a view to overcoming these difficulties; particular mention should be made of that of Allport and Skrimshire (1932) and that of the S.P.A. (1935) Sub-Committee for the detection of arsenic, lead, and other poisonous metals in food colouring-materials. The latter method was intended for use in connection with colouring-materials only, but it can be readily extended to all types of foodstuffs.

S.P.A. method for lead

REAGENTS

(1) Concentrated nitric acid—Sp. gr. 1·42, strength approx. 70 per cent wt/wt.

(2) Dilute nitric acid—Concentrated nitric acid is mixed with an equal volume of distilled water.

(3) Dilute hydrochloric acid—Concentrated hydrochloric acid, sp. gr. 1·16, strength approx. 32 per cent wt/wt, is mixed with an equal volume of distilled water.

(4) Concentrated ammonia—Sp. gr. 0·880.

(5) Dilute ammonia—One volume of concentrated ammonia is mixed with two volumes of water.

(6) Very dilute ammonia—One volume of concentrated ammonia is mixed with 10 volumes of water.

(7) Concentrated sulphuric acid—Sp. gr. 1·84, strength approx. 95 per cent wt/wt.

(8) Alcohol–water mixture—One volume of industrial methylated spirits, 64° O.P., is mixed with two volumes of distilled water.

(9) Alcohol–water–sulphuric acid mixture—10 volumes of industrial methylated spirit, 20 volumes of water and 1 volume of concentrated sulphuric acid are mixed together.

(10) Ammonium acetate solution—100 g of ammonium acetate are dissolved in 100 ml of distilled water.

(11) Dilute ammonium acetate solution—10 g of ammonium acetate are dissolved in distilled water and diluted to 100 ml.

(12) Potassium cyanide solution—10 g of potassium cyanide are dissolved in distilled water and diluted to 100 ml.

(13) Ammonium citrate solution—10 g of ammonium citrate are dissolved in distilled water and made up to 100 ml.

(14) Sodium sulphide solution—10 g of pure white sodium sulphide crystals are dissolved in distilled water and diluted to 100 ml.

(15) Standard lead solution—A solution containing 0·01 mg of lead in 1 ml. 0·1 g of pure lead foil or 0·16 g of pure lead nitrate is dissolved in dilute nitric acid and diluted to 100 ml with dilute nitric acid. This solution contains 1 mg of lead in 1 ml and may be retained as a stock solution. When required for use, 5 ml of this solution are diluted with water to 500 ml.

(16) Congo-red papers—Sheet filter-paper is dipped in a 0·1 per cent aqueous solution of purified Congo-red and dried as rapidly as possible in a warm atmosphere free from chemical fumes.

(17) Filter-papers—Before use, the filter-papers are washed with dilute hydrochloric acid and then with distilled water.

Method

Five grams of the sample are placed in a 200-ml Kjeldahl flask made of resistance glass or silica; with a liquid, the contents of the flask are then evaporated to dryness on a boiling water-bath. Ten ml of 30 per cent nitric acid are then added cautiously and the mixture heated until vigorous action ceases; after cooling, 10 ml of concentrated sulphuric acid are gradually added and the heating continued until the liquid darkens in colour. Five ml of nitric acid (sp. gr. 1·42) are gradually added to the hot solution in small portions and the liquid is boiled down until no further darkening occurs. With some foodstuffs, further additions of small amounts of nitric acid may be necessary before the liquid becomes colourless. The mixture is cooled,[1] diluted with about 100 ml of distilled water, and warmed until solution is apparently complete.

If any insoluble matter is observed, the solution is filtered and the residue washed with hot water. The paper and residue are boiled with 20 ml of dilute hydrochloric acid, the solution filtered, the insoluble matter again boiled with distilled water, filtered, and washed with hot distilled water. The filtrate and washings are added to the original acid filtrate, and the residue discarded.

The combined filtrate and washings are transferred to a beaker and diluted to 150 to 200 ml with water. Concentrated ammonia is gradually added, with stirring, until the major part of the acid has been neutralised. The solution is cooled, and dilute ammonia is added, drop by drop, with stirring, until the solution just fails to change the colour of wet Congo-red paper. (If iron is present in appreciable amounts it acts as an indicator, the end-point being such that the solution is deep-yellow in colour and the whole of the iron is just held in solution.)

At this stage there must be no permanent precipitate. Hydrogen sulphide is passed through the solution for at least 15 minutes to reduce any iron present to the ferrous condition. The solution is quickly heated to about 50° C and, while still warm, very dilute ammonia is added until the solution just fails to react acid to Congo-red paper. The addition of ammonia at this stage must be made slowly and carefully, with constant stirring to avoid precipitation of iron, aluminium, calcium, etc. If the end-point is passed, the solution should be made just acid by the addition of a few drops of dilute hydrochloric acid and again brought to the neutral point to Congo-red. After standing for 15 minutes with occasional stirring, the solution is filtered through a folded filter-paper (similar to Whatman No. 1). The precipitate is washed twice with 10 ml of saturated aqueous solution of hydrogen sulphide.

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[1] At this stage, arsenic, if present, may be distilled off and determined as described under the S.P.A. method for Arsenic in Food Colouring Materials (*Analyst*, 1930, **55** 102).

The filter-paper and precipitate are then transferred to the original digestion flask, the beaker washed with hot dilute nitric acid, using 20 ml in all, and the washings added to the flask. One ml of concentrated sulphuric acid is added, and the contents of the flask are digested over a Bunsen flame, using an asbestos square support having a circular aperture of 3 cm diameter, until the paper and precipitate are completely oxidised. If oxidation is difficult, a further small quantity of nitric acid is added and the heating continued. This operation is repeated until the solution remains colourless and the sulphuric acid is slightly fuming. The contents of the flask are cooled, 5 ml of water added, and the solution again heated to fuming. A small amount of aluminium sulphate may be deposited at this stage if fuming is unduly prolonged. Should this deposition occur, its solution is effected by the addition of 5 ml of water and further careful heating to fuming.

After cooling, 15 ml of alcohol–water mixture are added, and the contents of the flask are thoroughly mixed and allowed to stand overnight.

The liquid is then filtered slowly (about one drop per second) with suction through a Gooch crucible packed with a filter-paper pulp,[1] and the flask and crucible are washed with two successive portions of 5 ml of a mixture of alcohol–water–sulphuric acid in the proportions 10, 20, 1, by volume. The filtrate may be retained for the determination of copper.

Ten millilitres of ammonium acetate are added to the digestion flask and boiled, care being taken that the liquid comes into contact with the whole of the interior surface of the flask. The solution is poured on to the Gooch crucible and very gentle suction applied, so that the liquid slowly passes through. The flask and crucible are then washed with 10 ml of hot dilute ammonium acetate solution, followed by hot distilled water, until the filtrate measures almost 50 ml. The filtrate is cooled, transferred to a 50 ml or 75-ml measuring-flask, and made up to the mark. The whole of the solution or an aliquot part is transferred to a 100-ml Nessler cylinder; 5 ml of ammonium citrate solution, 2 ml of ammonia, and 4 ml of potassium cyanide solution are added, and the solution made up to the mark.

A series of standard comparison solutions are prepared, each containing in the same volume (100 ml) the quantities of ammonium acetate, ammonium citrate, ammonia and potassium cyanide solutions used in the test, together with varying quantities of standard lead solution. The test solution and standards should be clear and colourless. A coloured solution indicates incomplete oxidation, and the solution must be rejected and the whole determination recommenced. Each solution is mixed with 2 ml of sodium sulphide solution and the test solution matched against the standard comparison solutions. The volume of the aliquot portion of the test solution taken for colorimetric determination of the lead should be such that the colour produced with sodium sulphide is matched by a standard comparison solution containing preferably about 8 to 12 ml, and in any case not more than 15 ml, of standard lead solution.

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[1] *Preparation of Gooch crucible*—About 0·2 g of Whatman No. 1 or similar filter-paper is covered with concentrated hydrochloric acid and rubbed with a glass rod until thoroughly disintegrated. After diluting with water, the mixture is poured into a Gooch crucible having a base of about 2 cm diameter, the pulp gently but firmly packed by pressing with a flattened end of a glass rod, and washed until free from acid, using suction.

In order to determine the lead in the reagents (which should be " lead-free "), a blank experiment is carried out on the same quantities of reagents as used in the test.

For routine determination of lead, and where the greatest accuracy is not required, the following method may be used.

Ten or 25 grams of the sample are carefully charred and then ashed in a muffle furnace at a temperature not exceeding 500° C. The ash is cooled and treated with a little concentrated hydrochloric acid, evaporated to complete dryness and extracted with two lots of 10 ml of $\frac{1}{4}$ per cent hydrochloric acid, the solution being filtered into a Nessler cylinder, the filter washed with water and the filtrate made up to 50 ml. A measured portion of this solution is treated with 10 ml of 10 per cent ammonium acetate, 10 ml of 10 per cent ammonium citrate, ammonia until alkaline, 1 ml of 10 per cent potassium cyanide, and 0·5 ml of 10 per cent sodium sulphide, and the resultant colour is matched against standards containing the same amounts of reagents as the test sample and varying amounts of the standard lead solution used in the previous process.

The purpose of the ammonium citrate is to prevent interference from phosphates and small amounts of iron; potassium cyanide, of course, prevents interference due to the presence of copper.

The presence of lead may be proved in another portion of the original $\frac{1}{4}$ per cent hydrochloric acid solution by adding hydrogen sulphide water, heating rapidly almost to boiling, and filtering at once. After washing with a little hydrogen sulphide water, the filter and residue are transferred to a small dish, ignited carefully at a very low temperature, the ash treated with a few drops of 1–1 nitric acid, evaporated to dryness, and extracted with 2 to 3 ml of boiling ammonium acetate solution previously rendered distinctly acid with acetic acid. The liquid is filtered through a very small filter into a test-tube and, without further dilution, is tested with a drop of potassium chromate solution, the production of a yellow turbidity, of course, indicating the presence of lead.

Determination of copper

This can be conveniently carried out by the method of Callan and Henderson (1929) as modified by Haddock and Evers (1932) which depends on the formation of a coloured copper salt when a solution of sodium diethyl-dithiocarbamate is added to an alkaline solution containing copper.

Organic matter can be destroyed by ashing (copper not being volatile) or preferably by digestion with nitric and sulphuric acids, as in the determination of lead. If the latter metal is also to be determined, copper can be conveniently determined in the filtrate from the lead sulphate (p. 527).

The following is a brief account of the method of Haddock and Evers.

A suitable quantity of the acidified solution containing copper is boiled with a little hydrogen peroxide, cooled, 2 g of citric acid added and then ammonia until the pH of the solution is more alkaline than 9. The volume of the solution is finally made to 70 ml. Ten ml of a 0·1 per cent solution of sodium diethyl-dithiocarbamate are added to the alkaline solution and the mixture is immediately extracted with four quantities of 2·5 ml of carbon tetrachloride added from a burette. If the last extraction is more than faintly coloured, continue the extraction with four further quantities of 2·5 ml of carbon tetrachloride. Adjust the final volume of the coloured carbon tetrachloride in a small stoppered cylinder to either 10 or 20 ml. Measure the colour of the mixed extracts in a 1-cm cell in a Lovibond tintometer and read off the amount of copper from a curve based on the tables given below, according to whether 10 or 20 ml have been required, using only the yellow components of the colour. If the colour exceeds 10 units, repeat the experiment on a smaller quantity of the original solution.

Table 25.1—Determination of copper (volume of carbon tetrachloride, 10 ml)
(Haddock and Evers)

Copper added (mg)	*Colour Lovibond units, yellow*
0·005	0·7
0·01	1·2
0·02	2·5
0·03	4·2
0·04	6·4
0·05	8·2

Table 25.2—Determination of copper (volume of carbon tetrachloride, 20 ml)
(Haddock and Evers)

Copper added (mg)	*Colour Lovibond units, yellow*
0·05	3·1
0·06	4·2
0·07	5·1
0·08	6·3
0·09	7·4
0·10	8·3

It is sometimes difficult to obtain a perfectly colourless solution on the addition of ammonia to the solution prepared by acid digestion; for this purpose, Sage and Stevens (1938) recommended boiling the colourless acid-digested mixture with 10 ml of a saturated solution of ammonium oxalate, previous to diluting with water and making alkaline with ammonia.

The only metal which in ordinary circumstances is likely to interfere is iron in the ferrous state, so that unless it is definitely known that any iron has already been oxidised, the solution should invariably be heated with hydrogen peroxide as directed; the citric acid prevents the precipitation of phosphates and of ferric iron. Bismuth also causes serious interference (Strafford, 1933), and if its presence is suspected it should be removed before the test is applied.

The presence of traces of copper in milk has frequently been stated to promote the development of "tallowy" flavours; considerable attention has therefore been given to methods of determining this metal. Sylvester and Lampitt (1935) have described the following method, which depends upon the extraction of copper from an acid solution by means of a chloroform solution of diphenyl-thiocarbazone and then colorimetric determination by means of sodium diethyl-dithiocarbamate.

REAGENTS

(1) Copper-free distilled water.

(2) Copper-free 6N hydrochloric acid, prepared from acid distilled from glass apparatus.

(3) Diphenyl-thiocarbazone reagent—0·15 per cent in chloroform, prepared without heating and freed from copper as follows. Place 100 ml of chloroform solution and 100 ml of water in a separating-funnel and shake with 5 ml of 0·88 ammonium hydroxide. Discard the chloroform layer and wash the aqueous layer twice with 5 ml of chloroform. Add 200 ml of A.R. chloroform and hydrochloric acid until the aqueous layer after shaking is colourless. Run off the chloroform solution and store in a brown glass bottle.

(4) Sodium diethyl-dithiocarbamate reagent—Dissolve 0·1 per cent in cold distilled water and filter into a brown glass bottle. Prepare fresh each week.

(5) Standard copper solution—Contains 1·964 g of crystallised copper sulphate and 2 or 3 drops of concentrated sulphuric acid per litre. For use, dilute 50 times to give a dilute standard solution of which 1·0 ml ≡ 0·01 mg copper ≡ 1·0 p.p.m. on a 10-g sample.

(6) 5N ammonium hydroxide, prepared by diluting 0·88 NH_4OH with copper-free distilled water.

METHOD

Evaporate 20 g of milk to dryness with a few drops of dilute sulphuric acid and ash the residue at approximately 500° C in the presence of 2 ml of A.R. sulphuric acid. Dissolve the ash in 5 ml of 6N hydrochloric acid and heat to boiling. Dilute with 10 ml of water and heat to boiling, ensuring complete solution of the ash. After cooling, wash into a separating-funnel with two lots of 10 ml of water.[1] Add 5N ammonium hydroxide carefully until the solution is just neutralised and then add 1 ml of 6N hydrochloric acid. Extract the copper from this solution by shaking vigorously with three quantities of 5 ml of diphenyl-thiocarbazone reagent or, when necessary, until the colour in the 5 ml portion in use is not affected. (A reddish-purple colour appears in the chloroform layer when sufficient copper is present.) Wash the extracts with 10 ml of water, using the same water for successive washings of the separate chloroform extracts.

Combine the extracts in a pyrex boiling-tube (about 8 × 1 in.) and distil off the chloroform. Add 1 ml of A.R. concentrated sulphuric acid and 3 or 4 drops of 60 per cent A.R. perchloric acid. Boil until colourless. (The addition of a little more perchloric acid may be necessary when large amounts of diphenyl-thiocarbazone reagent have been used.) Dilute the residue with about 15 ml of water. Make the solution just alkaline with 5N ammonium hydroxide (about 7 ml) and, after cooling, pour into a 100-ml Nessler cylinder, rinsing the tube with water until the volume is about 50 ml. Prepare a blank solution in a second cylinder (which must match the first cylinder exactly), using 2·5 g of A.R. ammonium sulphate and water up to 50 ml. Add 3 ml of 5N ammonium hydroxide to each cylinder and 5 ml of sodium diethyl-dithiocarbamate reagent. Add dilute standard copper solution from a micro-burette to the blank cylinder until the yellow colour is not quite matched. Adjust the liquid levels in the tubes so that they are identical, and add to each an amount of A.R. amyl alcohol depending on the amount of copper present. Ten ml are sufficient for copper contents up to 1·5 ml of standard copper solution. Shake each cylinder vigorously, covering the top with the palm of the hand, and allow to settle. Observe the colour of the upper amyl alcohol layer by viewing horizontally, and match exactly by the addition of further standard copper solution, shaking both cylinders after each addition. Correct for the copper content of the reagents by making a control determination from the beginning to the end.

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[1] When the copper content is known to be high, an aliquot portion of the solution may be taken, containing preferably not more than 0·05 mg of copper.

None of the ordinary metals interferes with the determination. Sylvester and Lampitt give the natural copper content of 16 samples of milk drawn directly into glass bottles as varying between 0·09 to 0·17 p.p.m. with an average of 0·12 p.p.m.

Perrin *et al.* (1951) have described improvements in the carbamate method for estimating copper and the thiocyanate method for iron in dairy products. A special filtration procedure enables the two metals to be determined on the same sample and a large number of results are reported by these authors.

Other methods of determining copper in milk have been described by Supplee and Bellis (1922), Hess *et al.* (1923), and Conn *et al.* (1935).

Determination of tin

This metal may be determined by the method given on p. 517 for tin in wrapped cheese. With liquid products it is advisable to evaporate to dryness before adding sulphuric acid. All products containing fat should be charred in an open dish in the presence of sulphuric acid before being transferred to the Kjeldahl flask. Non-fatty substances can be weighed directly into a Kjeldahl flask and digested with potassium sulphate and sulphuric acid without preliminary charring, although, even in these cases, the latter is advisable.

Determination of zinc

This metal is frequently present in traces in foods, including ice-cream and other dairy produce, as a result of the use of galvanised containers. Its determination is therefore a matter of some importance, although it is by no means easy to obtain complete separation from other metals which may be present. Sylvester and Hughes (1936) have adapted the method of Lang (1929) and have suggested the following specific process for amounts of zinc not exceeding 0·3 mg.

(1) *Method for small amounts of zinc.* Ash a suitable quantity of the sample in a silica dish in a muffle furnace at 500° to 550° C. Treat the ash with 5 ml of 5N hydrochloric acid, heat to boiling, dilute with 10 ml of water and boil again. Cool, wash into a separating-funnel with 10 ml of water, and add 10 ml of 5N ammonium acetate solution.

Shake vigorously with 5 ml of diphenyl-thiocarbazone reagent (see " Determination of copper ", above) and transfer the chloroform extract to a second separating funnel. Wash the extract with a mixture of 6 ml of 5N ammonium acetate solution, 3 ml of 5N hydrochloric acid and 10 ml of water. Transfer the chloroform layer to a third separating-funnel and wash with 20 ml of distilled water. Finally, transfer the chloroform layer to a fourth separating-funnel, leaving the wash-waters in the second and third funnels.

Extract the liquid in the first funnel with a further 5 ml of diphenylthiocarbazone reagent and repeat the above procedure. Extract with further portions of reagent until its colour ceases to change. To the combined extracts add 10 ml of 0·5 N hydrochloric acid and shake. Transfer the acid layer to a 100-ml Pyrex beaker and wash the funnel with 10 ml of distilled water which is then added to the beaker. Re-extract the chloroform layer with another 10 ml of acid and again wash the funnel with 10 ml of water.

Evaporate the combined acid extracts in the beaker to dryness. Add 5 drops of pure perchloric acid and 5 drops of 10 vol. hydrogen peroxide and take to dryness on a hot-plate. Repeat until a white residue is obtained. Wash down the sides of the beaker with distilled water and again evaporate to dryness.

Add 0·1 ml of glacial acetic acid and about 0·01 g of ammonium hydrogen fluoride, followed by 2 ml of 5 per cent potassium iodide solution and 2 drops of 1 per cent starch solution. If a blue colour appears, add 0·002 N sodium thiosulphate until the colour is just discharged. Add about 0·5 ml of 1 per cent potassium ferricyanide solution and titrate with fresh 0·002 N sodium thiosulphate solution. The blue starch-iodide colour may be absorbed in the precipitated zinc ferrocyanide, and in this case the precipitate serves as an indicator.

0·51 ml of 0·002 N sodium thiosulphate solution $\equiv$ 0·10 mg of zinc $\equiv$ 10 p.p.m. on a 10 gram sample.

The above method is not affected by any of the other metals which may be extracted with zinc.

(2) *Method for larger amounts of zinc.* Where larger amounts are present the following method may be employed.

Ash 50 grams of the sample as above, moisten the ash with hydrochloric acid and evaporate to dryness. Extract twice with 10-ml quantities of boiling $\frac{1}{4}$ per cent hydrochloric acid, filter, and wash the filter and residue with distilled water. Precipitate Group 2 metals in the filtrate by the addition of H_2S water, heat almost to boiling, and filter. Oxidise the filtrate with nitric acid, add ammonium chloride, ammonia and acetic acid; boil, and filter off the precipitated phosphates of iron and aluminium. Pass sulphuretted hydrogen into the filtrate for 30 min. and coagulate the zinc sulphide on the water-bath. Filter, wash with H_2S water, dissolve in dilute hydrochloric acid and boil to remove hydrogen sulphide, neutralise with ammonia, add ammonium chloride and make just acid with hydrochloric acid; heat to boiling and titrate with standard potassium ferrocyanide solution (1 ml $\equiv$ 0·001 g zinc), using uranium acetate as external indicator.

Alternatively, the zinc may be determined nephelometrically in the solution as prepared for the above titration, by adding a small excess of potassium ferrocyanide solution, allowing to stand for five minutes, and comparing the turbidity with standards of exactly the same volume which contain exactly the same amounts of all reagents. Comparison is facilitated if the Nessler cylinders containing the test sample and standards are placed against a dark background and viewed by daylight reflected from above.

Determination of aluminium (Stafford and Wyatt, 1947)

Five or ten grams of the sample are treated with a mixture of 6 ml concentrated nitric acid, and 4 ml concentrated sulphuric acid in a 100-ml Kjeldahl flask. The mixture is warmed cautiously to start the reaction, and when this has passed the initially vigorous stage the solution is made to boil rapidly until it begins to darken. Nitric acid is added in small portions, until on further heating the solution no longer darkens but becomes a pale yellow colour. At this stage 0·5 ml perchloric acid and a little nitric acid are added and the mixture fumed for about 15 minutes. A further 0·5 ml perchloric acid is added, the mixture again heated for a few minutes, and allowed to cool. Ten ml water are added, the mixture boiled until it fumes, cooled, and 5 ml water added, and again boiled down until fumes appear.

The solution is then diluted until it is 5N to 6N in sulphuric acid, and transferred to a 50-ml separating funnel with the minimum amount of wash water. The solution should have a volume of 20 to 25 ml at this stage, and should contain not more than 10 mg of iron. The iron is removed in the following manner. 2·5 ml of a 6 per cent aqueous solution of cupferron are added and mixed well, and 10 ml of chloroform run in. After shaking vigorously for 40 seconds, the liquids are allowed to separate and the chloroform run off and discarded. The drops of strong iron " cupferate " solution remaining on the surface of the aqueous layer are displaced by the addition of a few ml of chloroform, and are then run off. The solution is next shaken with 5 ml chloroform for 30 seconds, allowed to separate, and the chloroform run off. The addition of 0·5 ml of the cupferrous solution at this stage should merely cause a white turbidity without the production of any colour due to iron. The cupferron is then removed by extraction with one 10-ml and two 5-ml portions of chloroform. Care should be taken that the final acidity of the solution is not below 4N or traces of aluminium may tend to be extracted.

The aqueous layer is transferred to a 100-ml conical flask and boiled down to a volume of 20 ml. After cooling, the solution is made just alkaline with 10N ammonium hydroxide, using one drop of methyl red as indicator. Hydrochloric acid, 5N, is added, drop by drop, until the solution is just acid, and then 1 ml in excess; 2 drops of saturated bromine water are added, and this should immediately bleach the indicator, failure to do so showing that the excess of cupferron has not been extracted completely. The excess of bromine is then reduced by the addition of 0·5 ml 10 per cent hydroxylamine hydrochloride solution.

The volume of the solution is adjusted to 30 ml, 1 ml 5 per cent gum arabic solution, 5 ml ammonium acetate buffer solution, and 2 ml of 0·2 per cent ammonium aurintricarboxylate solution added, and the mixture boiled for 5 minutes. After cooling to room temperature, 4 ml of 0·8 N ammonium borate solution is added, the solutions mixed, diluted to 50 ml and allowed to stand for at least 5 minutes before reading on the Spekker photoelectric absorptiometer. The optical density of the test solution and of a reagent blank solution are determined, using a 4-cm cell for the range 0 to 10 mg of aluminium and the 1-cm cell for quantities between 10 and 70 mg. Either Chance blue-green glass filters No. 6 (082) or Ilford green filters No. 604 should be used.

The standard curve is established by developing the colour of solutions containing measured volumes of a standard aluminium solution (1 ml = 10 mg of Al) and 1 ml of 5N hydrochloric acid in 30 ml in the manner detailed above. The optical density of each solution is determined against the reagent blank.

Strafford and Wyatt (1947) quote the following typical calibration figures, using a 1-cm cell—

Al present, mg	10	20	30	40	50	60	70
Optical density—							
(1) Ilford green No. 604 filters	0·14	0·285	0·43	0·57	0·72	0·86	1·00
(2) Chance blue-green No. 6 filters	0·10	0·20	0·305	0·405	0·51	0·62	0·71

REAGENTS (Strafford *et al.* 1945; Strafford and Wyatt 1943)

Care must be taken to reduce the reagent blank to as low a limit as possible and to avoid contamination during the test. The apparatus must be cleaned with hot 1 : 1 hydrochloric acid, followed by thorough washing with distilled water before use. The bromine water must be prepared by distillation of a saturated solution in an all-glass apparatus and collected in well cooled distilled water.

The ammonium acetate buffer solution, approximately 2N, is prepared by dissolving 156 g ammonium acetate, A.R., and 108 g ammonium chloride, A.R., in water, filtering, and making up to 1 litre.

The ammonium borate solution, 0·8N, is prepared by dissolving 93 g of powdered boric acid in 1 litre of N ammonium hydroxide, followed by filtration and dilution to 0·8N. The solution is standardised by diluting 25 ml to 150 ml and titrating with N HCl, using methyl red as indicator.

Further details are given in the original papers.

Determination of iron

Methods for iron and copper are described by McDowell (1947).

CHAPTER 26

STATUTORY TESTS FOR MILK

There are no standards, chemical or bacteriological, for ordinary milk sold " as such " apart from the legal presumptive standards of 3 per cent fat and 8·5 per cent solids-not-fat. It is illegal of course to add to, or subtract anything from, milk and therefore adulteration with water is an offence. This produces the curious anomaly that the seller of a naturally rich milk to which a small amount of water has gained access may be convicted for adulteration, but the seller of a milk which is naturally below the legal presumptive standards of either fat or solids-not-fat cannot be convicted if the milk is genuine although, of course, a buyer can refuse to accept the milk. There are no bacteriological standards of any description for ordinary milk, those laid down in the Milk (Special Designations) Order applying only to the designated milks.

Chemical standards and tests

The presumptive standards of 3 per cent fat and 8·5 per cent solids-not-fat apply to all types of milk sold in England and Wales as milk. If the milk is sold as " Channel Island milk " (sometimes referred to as " Jersey milk ") there is a standard of 4 per cent fat which is laid down in a Statutory Instrument. South Devon milk has now been included in this category and must similarly have a minimum fat content of 4 per cent. Both these milks qualify for a special premium of 4d. per gallon.

There are no legally defined methods for the determination of fat and solids-not-fat. In practice, milks are almost invariably tested by the Gerber method for fat and by the Richmond lactometer or the B.S.I. hydrometer for specific gravity or density, the total solids or solids-not-fat thus being worked out easily by the use of an appropriate slide rule or from tables. It is most important that these two methods should not be confused as the techniques and formulae are appreciably different. With careful working it may be assumed that the error of the Gerber method does not exceed 0·1 per cent fat and that of the lactometer or hydrometer 0·2 per cent solids-not-fat. Usually, of course, the error is less than this, and more accurate tests are only applied when values are obtained of less than 3 per cent fat and 8·5 per cent solids-not-fat. When a suspected milk is sent to a public analyst the fat is usually determined by the Röse-Gottlieb method and the solids-not-fat by making a gravimetric determination of the total solids and subtracting the fat. The genuineness of the milk, as distinct from its composition, is, in the United Kingdom, almost invariably checked by the determination of the freezing point.

Bacteriological tests

There are no bacteriological standards or tests laid down for the non-designated milks. The designations " Accredited " and " Tuberculin Tested " are subject to a special type of methylene blue test (commonly referred to as the "$4\frac{1}{2}/5\frac{1}{2}$ hour test") which must be carried out as follows.

TEST FOR ACCREDITED AND TUBERCULIN TESTED MILKS

Taking of samples

(1) Samples shall be taken at any time—

(*a*) when the milk (whether the special designation is used in relation to that milk or not) is in the possession of the licensed producer;

(*b*) when the milk is in the possession of the licensed dealer and the special designation is being used in relation to that milk by him.

(2) When the milk is in containers not exceeding one quart in capacity the sample shall consist of one such container which shall be delivered intact to the testing laboratory.

(3) When the milk is in containers exceeding one quart in capacity it shall be thoroughly stirred before sampling. The sample shall be taken from well below the surface of the milk. The instruments used for stirring and sampling shall be sterile.

(4) The sample shall be poured into a sterile bottle which shall thereupon be immediately stoppered. The part of the stopper which may come into contact with the milk shall be sterile.

Transport and keeping of samples

(5)—(*a*) The bottle or container containing any sample of milk to which sub-paragraph C. 4 (*b*) of Part I or sub-paragraph B. 5 (*b*) of Part II of the Second Schedule applies, shall be transferred forthwith to an insulated container, which shall not be artificially cooled, for transport to the laboratory. The sample shall be transported to the testing laboratory with the least possible delay. Upon arrival at the laboratory the sample shall be kept at atmospheric shade temperature until 6 p.m. on the day of production where the sample is from a morning milking, or until 10 a.m. on the day following production where the sample is from an evening milking; provided that for this purpose a sample of mixed milk from a morning milking and the evening milking of the previous day shall be regarded as a sample from the morning milking, and a sample of mixed milk from two milkings on one and the same day shall be regarded as a sample from the evening milking.

(*b*) Where the test of a sample to which this paragraph refers cannot be immediately begun at the end of the period for which the sample is to be kept, the sample shall be cooled to and kept at a temperature between 32° F and 40° F for a further period not exceeding 24 hours and the test shall be begun at the end of that period.

(6)—(*a*) The bottle or container containing any sample of milk other than a sample to which the preceding paragraph refers shall, save where the testing laboratory is situated on the premises where the sample is taken, be transferred forthwith to an insulated container, which shall not be artificially cooled, for transport to the testing laboratory: provided that unless the sample is to be delivered to the testing laboratory within two hours of the time of sampling, the sample shall be well packed in ice in a carrying box for transport to that laboratory. Samples shall be transported to the testing laboratory with the least possible delay.

(*b*) On arrival at the testing laboratory any such sample of milk shall be removed from the insulated container or iced carrying box, as the case may be, and, if the test is not then immediately begun, the milk shall be cooled to and kept at a temperature between 32° F and 40° F for a period not exceeding 24 hours and the test shall be begun at the end of that period. Where any such sample does not arrive at the testing laboratory on the day on which it is taken it shall be discarded unless—

- (i) the licensing authority deal with the sample in accordance with the foregoing requirements as though it had arrived at that laboratory on the day on which it was taken, and
- (ii) the sample is delivered to the testing laboratory on the day following that on which it was taken.

Identification of samples

(7) For the purpose of the identification of the sample in the laboratory the person taking the sample shall mark it with a number or other suitable identification mark and shall at the time of sampling enter in a book or on a paper, which shall accompany the sample, the following particulars—

- (*a*) the identification number or mark;
- (*b*) the name and address of the holder of the licence by whom the milk was consigned, or by whom it was being delivered, or on whose premises the sample was taken;
- (*c*) if known, the date of milking and whether milk of the morning or evening milking or mixed milk, and for this purpose milk produced before 12 noon on any day shall be treated as morning milk; milk produced at or after 12 noon on any day shall be treated as evening milk.

THE METHYLENE BLUE REDUCTION TEST FOR RAW MILKS

Reagent

(1) Methylene blue tablets manufactured under arrangements made by the Minister of Food shall be used for the test. A solution shall be prepared by adding one tablet to 200 ml of cold, sterile, glass-distilled water in a sterile flask, and by shaking until the tablet is completely dissolved and making up the solution to 800 ml with cold, glass-distilled water. The resultant solution shall be stored in a stoppered flask in a cool, dark place, and shall not be used if—

- (*a*) it has been exposed to sunlight; or
- (*b*) a period of two months has elapsed since the date of preparation.

(2) The amount of methylene blue required for a day's work shall be poured off from the stock bottle into a suitable glass container. The pipette used for transferring the methylene blue solution to the tubes of milk shall not be introduced into the stock bottle.

APPARATUS

(3)—(*a*) Test tubes shall conform to the British Standard Specification No. 625 (1935) 152/16 nominal 6 in. × $\frac{5}{8}$ in. having an internal diameter of 13·5 ± 0·5 mm and being accurately marked at 10 ml. They shall be plugged with cotton wool, or covered with closely fitting aluminium caps, or stored in such other way as may prevent contamination.

(*b*) Pipettes shall be 1·0 ml straight-sided blow-out delivery pipettes and shall be plugged with cotton wool at the upper end.

(*c*) Glassware, and rubber stoppers, shall be sterile before use.

METHOD OF CARRYING OUT THE TEST

(4) The sample of milk shall be thoroughly mixed by inverting and shaking the sample bottle and the milk shall then be poured into a test tube up to the 10 ml mark, leaving one side of the interior unwetted with milk. One ml of methylene blue solution shall be added without letting the pipette come into contact with the milk in the tube or with the wetted side of the interior of the tube. After a lapse of 3 seconds, the solution remaining in the tip of the pipette shall be blown out. The tube shall be closed with a rubber stopper with aseptic precautions. The tube shall then be slowly inverted twice so that the whole column of contained air rises above the level of the milk, and placed within 5 minutes in a water bath. The water in the bath shall be kept above the level of the milk in the test tubes, and its temperature, which shall be between 37° C and 38° C, shall be maintained as nearly uniform as possible by means of a reliable automatic thermo-regulator. The interior of the bath shall be kept completely dark.

(5) To indicate when decolorisation is commencing, and when it is complete, two control tubes shall be used for comparison with each batch of tubes containing the milk under test. One control tube shall be prepared by immersing in boiling water for not less than three minutes a properly plugged test tube containing 1 ml of tap-water and 10 ml of a mixture of milk having a fat content and colour similar to that of the milk being tested, and a second control tube shall be prepared by immersing in boiling water for not less than 3 minutes a properly plugged tube containing 1 ml of methylene blue solution and 10 ml of a mixture of milk having a fat content and a colour similar to that of the milk being tested.

(6) The tubes containing the milk under test and the control tubes shall be inspected at half-hourly intervals. At these inspections—

(*a*) any tube in which the milk has become decolorised shall be removed from the water bath;

(*b*) any tube in which decolorisation has begun shall remain without inversion in the water bath until decolorisation is complete; and

(*c*) all other tubes in the water bath shall be inverted once and replaced.

(7) The time, within the limits of 4½ hours to 5½ hours, as the case may be, at which decolorisation is observed, shall be recorded.

(8) The milk shall be regarded as decolorised when the whole column of milk is completely decolorised or is decolorised up to within 5 mm of the surface. A trace of colour at the bottom of the tube may be ignored provided that it does not extend upwards for more than 5 mm.

INTERPRETATION

(9) A sample shall be regarded as satisfying the methylene blue reduction test if, between 1st May and 31st October, it fails to decolorise the methylene blue in 4½ hours or if, between 1st November and 30th April, it fails to decolorise the methylene blue in 5½ hours.

TESTS FOR PASTEURISED MILKS

Pasteurised milk is subjected to a different form of the methylene blue test, commonly referred to as the " half-hour methylene blue test ", which must be carried out as follows.

Taking of samples

(1) Samples shall be taken at any time—

(*a*) when the milk (whether the special designation is used in relation to that milk or not) is in the possession of the pasteuriser or steriliser, as the case may be;

(*b*) when the milk is in the possession of the licensed dealer and the special designation is being used in relation to that milk by him.

(2) When the milk is in containers not exceeding one quart in capacity the sample shall consist of one such container which shall be delivered intact to the testing laboratory.

(3) When the milk is in containers exceeding one quart in capacity it shall be thoroughly stirred before sampling. The sample shall be taken from well below the surface of the milk. The instruments used for stirring and sampling shall be sterile.

(4) The sample shall be poured into a sterile bottle which shall thereupon be immediately stoppered. The part of the stopper which may come into contact with the milk shall be sterile.

Transport of samples

(5) Save where the testing laboratory is situated on the premises where the sample is taken, the bottle or other container containing the sample shall be transferred forthwith to an insulated container, which shall not be artificially cooled, for transport to the testing laboratory.

(6) Samples shall be transported to the testing laboratory with the least possible delay and shall be delivered there on the day on which they are taken. If the sample does not arrive on the same day it shall be discarded.

Identification of samples

(7) For the purpose of the identification of the sample in the laboratory the person taking the sample shall mark it with a number or other suitable identification mark and shall at the time of sampling enter in a book or on a paper, which shall accompany the sample, the following particulars—

(*a*) the identification number or mark;

(*b*) the name and address of the holder of the licence by whom the milk was consigned, or by whom it was being delivered, or in whose premises the sample was taken.

THE METHYLENE BLUE TEST FOR PASTEURISED MILK

(1) On arrival at the laboratory, the samples of milk shall be removed from the insulated container and kept at atmospheric shade temperature until the test is begun. If at any time the atmospheric shade temperature in the immediate vicinity of the samples, as indicated by a maximum thermometer adjusted to below 65° F at 9 a.m. on each day of sampling, has exceeded 65° F, the tests shall be void. Samples shall not be kept in a refrigerator or cold store or water bath or in an incubator. Tests shall be begun between 9 and 10 a.m. on the day after the samples are taken.

REAGENT—METHYLENE BLUE

(2)—(i) Tablets manufactured under arrangements made by the Minister shall be used for the test. A solution shall be prepared by adding one tablet to 200 ml of cold, sterile glass-distilled water in a sterile flask, shaking until the tablet is completely dissolved, and making up the solution to 800 ml with cold glass-distilled water. The resultant solution shall be stored in a stoppered flask in a cool, dark place, and shall not be used if—

(*a*) it has been exposed to sunlight, or
(*b*) a period of two months has elapsed since the date of preparation.

(ii) The amount of methylene blue required for a day's work shall be poured off from the stock bottle into a suitable glass container. The pipette used for transferring the methylene blue solution to the tubes of milk shall not be introduced into the stock bottle.

APPARATUS

(3)—(i) Test tubes shall conform to the British Standard Specification No. 625 (1935) 152/16, nominal 6 in. × $\frac{5}{8}$ in., having an internal diameter of 13·5 ± 0·5 mm and being accurately marked at 10 ml. They shall be plugged with cotton wool, covered with closely fitting aluminium caps or stored in such other way as may prevent contamination.

(ii) Pipettes shall be 1·0 ml straight-sided blow-out delivery pipettes, and shall be plugged with cotton wool at the upper end.

(iii) Glassware and rubber stoppers shall be sterile.

METHOD OF CARRYING OUT THE TEST

(4)—(i) The test shall be carried out in the following manner—

Thoroughly mix the sample of milk by inverting and shaking the sample bottle and pour the milk into a test tube up to the 10 ml mark, leaving one side of the interior unwetted with milk. Add 1 ml of methylene blue solution without letting the pipette come into contact with the milk in the tube or with the wetted side of the interior of the tube. After a lapse of 3 seconds, blow out the solution remaining in the tip of the pipette. Close the tube with a rubber stopper with aseptic precautions. Invert the tube slowly twice, so that the whole column of contained air rises above the level of the milk, and place within 5 minutes in a water bath. The water in the bath shall be kept above the level of the milk in the test tubes; and its temperature, which shall be between 37° C and 38° C, shall be maintained as nearly uniform as possible by means of a reliable automatic thermo-regulator. The interior of the bath shall be kept completely dark.

(ii) To indicate when decolorisation is complete a control tube shall be used for comparison with each batch of experimental tubes. The control tube shall be prepared by immersing in boiling water for three minutes a stoppered test tube containing 1 ml of tap water and 10 ml of mixed milk having a fat content and colour similar to that of the milk being tested.

(iii) The milk shall be regarded as decolorised when the whole column of milk is completely decolorised or is decolorised up to within 5 mm of the surface. A trace of colour at the bottom of the tube may be ignored provided that it does not extend upwards for more than 5 mm.

INTERPRETATION

(5) The test shall be deemed to be satisfied by milk which fails to decolorise methylene blue in thirty minutes.

Although not strictly speaking a bacteriological test the phosphatase test is now legally defined as the statutory method for testing the efficiency of pasteurisation. The modification of the phosphatase test which is now laid down is as follows—

THE PHOSPHATASE TEST FOR PASTEURISED MILK

(1) Samples of the milk shall be examined as soon as possible after arrival at the laboratory. If they are not examined immediately on arrival at the laboratory they shall be kept at a temperature of between 32° F and 40° F until examined. All samples shall be raised to room temperature immediately before being tested.

PRECAUTIONS

(2) The following precautions shall be taken—

(*a*) Samples which show a taint or clot on boiling shall not be tested.
(*b*) Phenols, disinfectants and detergents containing phenols, and soap containing carbolic acid shall be kept apart from the test reagents and apparatus.
(*c*) Bottle caps made from phenolic resins shall not be used.
(*d*) Rubber stoppers shall not be used until they have been shown by test not to contain phenolic impurities.
(*e*) All glassware shall be clean before use.
(*f*) A fresh pipette shall be used for each sample of milk. Pipettes shall not be contaminated with saliva.
(*g*) All reagents shall be kept in a cool dark place and shall be well protected from dust.
(*h*) Tests shall not be carried out in direct sunlight.
(*i*) Freshly boiled distilled water shall be used throughout.

REAGENTS

(3)—(i) Whenever possible reagents of analytical quality should be used.

(ii) *Buffer substrate.* Solutions shall be prepared either—

(*a*) by dissolving 1·09 g of disodium phenyl phosphate and 11·54 g of sodium veronal (sodium diethyl barbiturate) in distilled water saturated with chloroform, and by making up to one litre; or

(*b*) by adding one buffer-substrate tablet to about 45 ml of boiling distilled water, boiling for exactly one minute, cooling rapidly, making up to 50 ml with boiled distilled water and adding a few drops of chloroform.

The buffer-substrate solution shall be kept in a refrigerator. It may be used for a period not exceeding three days from the date of preparation.

(iii) *Folin and Ciocalteu's phenol reagent* (*stock*). The reagent to be used shall be manufactured under arrangements made by the Minister or shall be prepared in the following manner—

Dissolve 100 g of sodium tungstate $Na_2WO_4 . 2H_2O$ and 25 g of sodium molybdate $Na_2MoO_4 . 2H_2O$ in 700 ml of distilled water in a 1,500-ml flask connected to a reflux condenser by a ground glass joint, or, if that is not available, by a rubber stopper or cork wrapped in tin foil, provided that the solution shall not come into contact with the tin foil. Add 50 ml of syrupy (85 per cent) phosphoric acid and 100 ml of concentrated hydrochloric acid. Reflux the mixture gently for 10 hours, then cool, add 150 g of pure lithium sulphate, 50 ml of distilled water and from 4–6 drops of liquid bromine and leave for two hours. Then remove the excess bromine by boiling the mixture under the hood without the condenser for 15 minutes. Cool, dilute to 1 litre with distilled water, and filter. If the finished reagent has a greenish tint it shall be rejected; if it has a golden-yellow colour with no greenish tint it shall be considered fit for use. The reagent shall be stored in a refrigerator and be protected from contact with dust, metal or any reducing substance. After four months from the date of preparation any remaining reagent shall be discarded and a fresh stock reagent prepared.

(iv) *Sodium hexametaphosphate.* The salt shall be used in the form of flakes. It shall be kept in a tightly stoppered bottle. If any white powder has formed during storage, the salt shall not be used until that powder has been removed by sifting. A 5 per cent solution (weight per volume) shall be prepared by dissolving in warm water and making up to volume after cooling.

(v) *Test reagent.* The test reagent shall be prepared by adding one volume of the stock solution of Folin and Ciocalteu's reagent to two volumes of the hexametaphosphate solution. If any precipitate appears in it, or in any case after four months from the date of preparation, any remaining test reagent shall be discarded and a fresh test reagent prepared.

(vi) *Sodium carbonate.* A 14 per cent solution (weight per volume) shall be made up of anhydrous sodium carbonate of analytical reagent quality.

APPARATUS

(4) The following apparatus shall be used—

(*a*) A water bath or incubator capable of being maintained at 37 ± 1° C.

(*b*) A pipette or an automatic burette to deliver 4·5 ml. The latter shall be made from dark glass or be painted a dark colour.

(*c*) A supply of 1·0-ml straight-sided pipettes of an accuracy equal to that of N.P.L. grade B, marked 0·5 and 1·0 ml and plugged with cotton wool.

(*d*) A supply of test tubes conforming to British Standard Specification No. 625 (1935) 152/16, accurately marked at 10 ml with rubber stoppers to fit.

(*e*) A supply of filter funnels, 5 cm diameter.

(*f*) A supply of Whatman filter papers, 9 cm No. 40.

(*g*) Either a Lovibond " all purposes " comparator with cell of 25 mm depth and disc containing standard coloured glasses corresponding to 0·5, 1·5, 2·3 and 6·0 Lovibond blue units; or a Lovibond tintometer with 13 mm cell.

CARE OF APPARATUS

(5)—(i) After use each test tube shall be well washed in hot water containing soda, rinsed in hot clean water and then washed with 50 per cent commercial hydrochloric acid. The acid wash shall be carried out by filling one tube with the acid and passing it from tube to tube, the acid being replenished when necessary. The tubes shall then be well rinsed again in hot clean water and dried.

(ii) New glassware shall be cleaned in chromic acid prepared in the following manner. Dissolve 90 g of powdered potassium bichromate in 200 ml of hot water contained in a four-litre conical flask. Cool, add two litres of commercial sulphuric acid (not less than 90 per cent). Stir until the precipitate has dissolved. Keep the solution covered and discard when it becomes green. After cleaning in chromic acid, new glassware shall be well rinsed in hot water and dried by evaporation.

(iii) Funnels shall be washed in hot clean water.

(iv) Pipettes shall be well rinsed in cold, clean water and shall be cleaned by soaking for 24 hours in chromic acid solution in a 250-ml glass cylinder or other suitable container.

(v) Glassware used for the test shall not be used for any other purpose, and shall be kept apart from all other apparatus in the laboratory.

METHOD OF CARRYING OUT THE TEST

(6) The test shall be carried out in duplicate in the following manner.

To 10 ml of the buffer-substrate solution contained in a test tube, add 0·5 ml of well mixed milk. Add 3 drops of chloroform, stopper the tube, mix the contents and incubate at 37 ± 1° C for 24 ± 2 hours. At the end of this time, cool, add 4·5 ml of the test reagent, mix, allow to stand for 3 minutes, and filter into a test tube marked at 10 ml. If an automatic burette is used for delivery of the test reagent and has stood full of reagent for more than 24 hours, the first two emptyings of the burette shall be discarded. To 10 ml of the filtrate add 2 ml of sodium carbonate solution, mix, and place the test tube for exactly 2 minutes in a bath of boiling water which must be kept boiling. Where a number of tubes are being examined, the period of two minutes shall be timed from the moment when the water has recommenced boiling. Cool and proceed to read the colour thus developed, using either the comparator or the tintometer.

Control tests

(7)—(i) All milk samples shall be kept in a refrigerator for 24 hours after the duplicate experimental tubes have been put into an incubator. When the test has been completed, control tests shall be carried out on all samples which have given a positive phosphatase reaction, in the following manner—

Mix thoroughly 10 ml of the buffer-substrate solution with 4·5 ml of the test reagent, add 0·5 ml of milk, and mix. Allow to stand for 3 minutes and filter into a test tube marked at 10 ml. To 10 ml of the filtrate, add 2 ml of the sodium carbonate solution, mix and place the tube for exactly 2 minutes in a bath of boiling water which must be kept boiling. Where a number of tubes are being examined, the period of two minutes shall be timed from the moment when the water has recommenced boiling. Cool and proceed to read the colour developed.

(ii) If the colour reading exceeds 1·5 Lovibond blue units, the test shall be void.

(iii) The colour reading obtained from the control test shall not be subtracted from the reading obtained from the incubated sample.

(8) The purity of the reagents shall be tested by incubating with each batch of samples a tube containing buffer substrate and chloroform but no milk. Proceed after incubation exactly as directed above under " Method of carrying out the test ". If the colour reading thus obtained exceeds 0·5 Lovibond blue units, the test for the particular batch of samples concerned shall be void.

Interpretation

(9) The test shall be deemed to be satisfied by milk which gives a reading of 2·3 Lovibond blue units or less.

TURBIDITY TEST FOR STERILISED MILK

An entirely new test has been introduced for sterilised milk. This is known as the *Turbidity Test* and is based fundamentally upon the fact that if milk is heated to at least 100° C for a short time, all the albumin becomes precipitated. The technique for this test is as follows—

(1) Samples may be examined at any time after delivery to the laboratory but shall be at room temperature when the test is begun.

Reagent

(2) Ammonium sulphate A.R. shall be used.

Apparatus

(3) The following apparatus shall be provided—

(*a*) A supply of conical flasks, 50 ml capacity.
(*b*) A supply of graduated cylinders, 25 ml capacity.
(*c*) A supply of test tubes conforming to British Standard No. 625—1935, 127/12.
(*d*) A supply of filter funnels, 6 cm diameter.
(*e*) Two beakers, 400 ml capacity.
(*f*) A supply of Whatman folded filter papers, 12·5 cm No. 12.

METHOD OF CARRYING OUT THE TEST

(4) The test shall be carried out in the following manner—

Weigh 4 ± 0·1 g of ammonium sulphate into a 50-ml conical flask. Measure out 20 ± 0·5 ml of the milk sample, and pour into the conical flask. Ensure that the ammonium sulphate dissolves by shaking for one minute. Leave for not less than five minutes and then filter through a folded paper into a test tube. When not less than 5 ml of a clear filtrate have collected, place the tube in a beaker of water, which is kept boiling, and keep it therein for five minutes. Transfer the tube to a beaker of cold water, and when the tube is cool, examine the contents for turbidity by moving the tube in front of an electric light shaded from the eyes of the observer.

INTERPRETATION

(5) The test shall be deemed to be satisfied by milk which shows no sign of turbidity.

CHAPTER 27

MISCELLANEOUS TESTS

As this book is concerned only with dairy chemistry detailed descriptions of the various miscellaneous tests which the dairy chemist may be called upon to perform are not given, but the following notes and references may be found useful.

For general reference Cronshaw's *Dairy information*, Ling's *A text book of dairy chemistry*, and Davis's *Milk testing* and the same author's *Dictionary of dairying* may be consulted.

Abnormal milk

See Mastitis milk, *below.*

Bacteriological tests

There are five main types of bacteriological tests—

(1) The plate count, in which various quantities of the milk or other material are mixed with a nutrient medium and the colonies which grow after a standard incubation are counted.

(2) Tests such as the presumptive coliform test and the milk-souring organisms test in which various quantities of the milk, etc., are added to a liquid medium in the tube and the presence of certain types of organisms detected after incubation by observing changes in the medium, e.g. gas, acid production, etc.

(3) Dye reduction tests such as the methylene blue and resazurin tests in which the reducing capacity of the growing organisms brings about a change in the colour of the dye.

(4) Titratable acidity, in which the acid produced by the growing organisms is titrated with a standard alkali.

(5) The direct microscope count in which a small amount of milk is smeared on a slide, dried and stained with methylene blue or some other suitable stain, the bacterial cells then being counted with a microscope.

Descriptions of these methods are given in Davis (1950 and 1953, 1951a) and also in Wilson *et al.*, *The bacteriological grading of milk*. For a recent discussion of the bacteriological grading of milk see Davis (1950b).

Chlorine

The requirements for concentrations of chlorine as laid down in various regulations and official documents may make it necessary for the dairy chemist to check the strength of hypochlorite solutions from time to time. No great accuracy is required in this work, and for ordinary purposes the Tintometer chlorine discs will be found to be quite satisfactory for the purpose. Two discs are available—the K.D.1 and the K.D.2 in different ranges of chlorine concentration. It is important to note, however, that for a quick and satisfactory result the solution must be below pH 6 and it is not always sufficient to add a phosphate buffer. It is recommended that three or four drops of acetic acid should be added to the solution when making chlorine estimations.

If a more accurate determination is required the ordinary iodide–thiosulphate method is suitable.

Detergent solutions

The high standards for cleanliness and sterility in dairy equipment make it most important that the strength of cleaning solutions should always be under the control of the laboratory. There are two main methods of checking the strength of detergent solutions—by titration with a standard acid and using an instrument such as a Detergent Concentration Indicator. The first measures the titratable alkalinity of the solution to phenolphthalein or methyl orange, according to the indicator used, and instruments are usually based upon the measurement of conductivity. There are so many aspects of detergent action that no one test gives all the information, but either of the two mentioned is satisfactory for individual purposes if properly used. (See Davis 1950 and 1953, and 1951b.)

Effluent

According to the nature of the dairy operations, the possible methods of effluent disposal and the requirements of local authorities, the control of effluent may be an important function of the dairy chemist. There are two main types of methods—the determination of the actual solids in the effluent, and the estimation of the " biological oxygen demand " which is an indirect measure of the amount of food material available in the effluent for bacteria. A rough estimation of milk solids in effluent may be made by comparing the turbidity of the effluent in a large round flask with standards set up by adding known amounts of milk to water. The standard method for estimating the biological oxygen demand is given in Min. Health Bulletin No. 70, H.M.S.O. See also the section on " Effluent " by Allen in Davis's *Dictionary of dairying*.

Mastitis milk

Mastitis is due to an infection of the udder and may exist in a variety of conditions from the barely detectable sub-clinical to the acute clinical in which the mammary secretion may contain blood and even be serous in nature. There is no necessary relation between any of the simple, indirect tests, the bacteriological tests and the chemical composition of the milk, but, broadly speaking, the more severe the mastitis the more closely does the composition of the mammary secretion approach that of serum. The most marked effect from the chemical point of view is the decrease in the lactose and the increase in the sodium chloride. There is moreover a decrease in the calcium and a rise in pH. The protein distribution is also affected, the percentage of casein falling and that of globulin rising. This is the basis of the " casein number ", a method which has been developed by S. J. Rowland and Zein-El-Dine (1939) for the chemical detection of mastitis or abnormal milk.

The increase in sodium chloride results in an increased conductivity of the milk in the diseased quarter and this forms the basis of the conductivity method for the quick detection of abnormal milk. It is important with this method to compare the *differences* between the values for the four quarters (Davis 1947). The normal range of conductivity for mastitis-free milk is 35 to 45 $\times$ 10^{-4} mhos. In late lactation milk alters chemically in much the same way as mastitis milk, i.e. the composition tends to approach that of serum. It follows therefore that in late lactation the conductivity of the milk from the four quarters will also rise, but with normal late-lactation milk the conductivities of the secretions of all four quarters are roughly the same. There is a high correlation between the conductivity and the leucocyte content of milks (Little and Plastridge, 1946; Munch-Petersen 1938; Malcolm *et al* 1942; Malcolm and Campbell 1946).

Sterility of equipment

Much attention has been paid recently to methods for assessing the sterility of equipment in dairy practice, and a standard of one colony per millilitre or square centimetre of surface has come to be generally accepted, although there are so far no legal standards in this respect. Methods are based upon either a swabbing of a given area or the rinsing of the container by a known amount of sterile quarter-strength Ringer solution.

Methods

(i) *Bottles and cartons.* The simplest method is to add 20 ml of Ringer solution and, after rinsing by standardised technique, plating suitable quantities, e.g. 5 ml and/or 1 ml (Min. Agric. 1947, B.743/T.P.B.).

(ii) *Cans (or " churns ").* The method is basically the same as that for bottles and consists in adding 500 ml of Ringer solution and rinsing the can by a standardised technique, 1 ml of the rinse being plated (Min. Agric. Form No C.168/T.P.Y.).

(iii) *Tanks, tankers, pipelines, pasteurising plants, fillers and other equipment.* The usual method is to swab a known area and squeeze out the swab in a known volume, e.g. 20 ml of Ringer solution, plating 1 ml (Min. Agric. Form No. C.195/T.P.Y.).

Water

The qualities required in a water will vary according to the type of operations, e.g. dairy farm, milk collecting depot, manufacturing creamery and pasteurising dairy. Broadly speaking, a water supply should be not too hard and bacteriologically of good condition. If the hardness exceeds 5 parts per 100,000 it is advisable to fit a water-softening plant. Of the bacteriological tests, by far the most useful is the presumptive coli test. Coli should be absent in 10 ml. A survey of the subject is given by Allen in the section on " Water supplies " in Davis's *Dictionary of dairying.*

For steam-raising the chemical properties of the water are of much more importance than the bacteriological, and it is financially well worth while to institute a proper chemical control of water for boilers (Dickinson 1950).

APPENDIX

PRESERVATION OF MILK SAMPLES

Where any special importance is attached to the analysis of a sample, it is an advantage to preserve the sample for reference and further corroborative analysis. Preservatives are added to effect this. The following substances have been used.

Alcohol

Allen suggested adding to the milk to be kept twice its weight of alcohol; his experience and that of Hehner show that analytical data can be obtained on the preserved milk (making allowance for the alcohol added) which agree with the original sample. The objection to this method is that a large amount of a volatile substance is added, and a correction, the exactness of which depends on the amount of alcohol present, must be made. Milk-sugar and salts are also deposited after some time, and are difficult of complete redistribution.

Chloroform

When added in the proportion of 1 ml to 100 ml of milk, chloroform keeps the milk for a short time. It has the advantage of dissolving in the fat and keeping the cream in an easily miscible condition. As Babcock and Russell have shown, it does not stop enzymic action; hence changes in the proteins, due to this cause, proceed as if no chloroform had been added. The correction to be applied is small. For keeping samples for a short period, say ten days, this method is good.

Ether

This preservative is nearly as good as chloroform; it is, however, not quite so effective and also it is more volatile.

Collins recommended a mixture of ether and chloroform of specific gravity 1·032, as it does not affect the specific gravity of the milk. Richmond showed, however, that ether and chloroform keep the fat in a liquid condition, and that the specific gravity is lowered by this cause. The estimation of the fat by the Gerber method is too high in the presence of chloroform.

Terpenes, thymol, dichlorophenol, and salicylic acid

These keep the milk, but allow the cream to rise to the surface, where it sets in a firm layer and is not easily redistributed.

Hydrofluoric acid and fluoboric acid

Richmond showed that these substances, when added to fresh samples in the proportion of ½ ml to 100 ml of milk, keep them in good condition, and after a year analysis gives the same figures as those previously found. They curdle the milk, however, so that the sample must be shaken well to bring the precipitated casein into a fine state of division; a little of the bottle is dissolved and the ash is thereby slightly increased. Richmond found this method to be one of the best.

Formalin

The addition of formalin has many advantages. Only a very minute amount of the 40 per cent solution need be added (2 drops per 100 ml), and no correction is necessary for so small a quantity.

Siegfeld found that the presence of much formaldehyde in milk has a tendency to increase the amount of fat by the Gerber method. This may be obviated by adding 1 ml of hydrogen peroxide, or better, 0·5 ml of a 40 per cent solution of hydroxylamine hydrochloride per 100 ml of milk, and correcting for increase of volume.

The formaldehyde, however, combines with the protein and raises the apparent percentage of total solids and solids-not-fat. Bevan suggested that the milk-sugar is hydrolysed into dextrose and galactose, as he found the increase in total solids more than the total amount of formaldehyde added; but this has been disproved by Höft.

Potassium bichromate, mercuric chloride, and solid antiseptics

These add considerably to the weight of the total solids and solids-not-fat, and cannot therefore be recommended. If fat only is to be determined they are efficient. Siegfeld does not consider that the analysis of samples preserved by potassium bichromate is trustworthy. Talenti and Sica (1948) have stated that potassium bichromate plus 1 per cent amyl alcohol are most effective if added directly after milking. In this way milk samples may be kept for six weeks. Gibbons and Brown (1948) state that chloropicrin (or microlysine) at a concentration of 50 to 75 p.p.m. kept milk fresh at room temperature for 3 to 5 days.

Sterilisation

This is sometimes resorted to; certain changes result which, however, do not usually interfere with the analysis. The cream rises and clots on the surface, and it is not easy to obtain an average sample.

Cold storage

Samples may be frozen and kept in a cold chamber, if one is available; they keep for an indefinite period thus, but require carefully remelting and remixing. This method, which is not always available, is superior to all others, and should be resorted to in those dairies which possess a freezing plant and cold-storage room.

Table A.1—Weights and measures: conversion table

Linear measure

1 inch = 0·0254 metre
1 foot = 12 inches = 0·3048 ,,
1 yard = 3 feet = 36 inches = 0·9144 ,,
1 metre = 10 decimetres = 100 centimetres = 1000 millimetres
1 metre = 39·371 inches = 3·281 feet = 1·094 yards

Square measure

1 square inch = 0·000645 sq. metre
1 square foot = 144 square inches = 0·0929 ,,
1 square yard = 9 square feet = 1,296 square inches = 0·8361 ,,
1 sq. metre = 100 sq. decimetres = 10,000 sq. centimetres = 1,000,000 sq. millimetres
1 sq. metre = 1550·6 sq. inches = 10·764 sq. feet = 1·196 sq. yards

Cubic measure

1 cubic inch = 0·00001639 cub. metre
1 cubic foot = 1,728 cubic inches = 0·0283 ,,
1 cubic yard = 27 cubic feet = 46,656 cubic inches = 0·7645 ,,
1 cubic metre = 1,000 cubic decimetres = 1,000,000 cubic centimetres
= 1,000,000,000 cubic millimetres
1 cubic metre = 61,027 cubic inches = 35·317 cubic feet = 1·308 cubic yards

Measures of capacity

1 gill = 0·1420 litre
1 pint = 4 gills = 0·5679 ,,
1 quart = 8 gills = 2 pints = 1·1359 litres
1 gallon = 32 gills = 8 pints = 4 quarts = 4·5435 ,,
1 litre = 1,000 cubic centimetres = 1,000,000 cubic millimetres
1 litre = 7·043 gills = 1·7608 pints = 0·8804 quarts = 0·2201 gallon

Avoirdupois weight

1 grain = 0·0648 gram
1 drachm = 1·7718 grams
1 ounce = 16 drachms = 28·349 ,,
1 pound = 256 drachms = 16 ounces = 453·59 ,,
1 quarter = 7,168 drachms = 448 ounces = 28 lb = 12,700·5 ,,
1 hundredweight = 28,672 drachms = 1,792 oz = 112 lb
= 4 quarters = 50,802 ,,
1 ton = 573,440 drachms = 35,840 oz = 2,240 lb = 80 qrs
= 20 cwt = 1,016,047 ,,

1 metric ton = 1,000 kilograms
1 kilogram = 1,000 grams
1 gram = 10 decigrams = 100 centigrams = 1,000 milligrams
1 gram = 15·43 grains = 0·5644 drachm = 0·03527 oz = 0·002205 lb
= 0·00001968 cwt = 0·000000984 ton

Useful data

The gallon weighs 10 lb (of distilled water at 62° F).
The litre weighs 1,000 grams (of distilled water at 0° C).
1 gallon = 4½ litres approximately
1 kilogram = 2¼ lb approximately
1 hundredweight = 50 kilograms approximately
1 cubic foot = 6·24 gallons

Table A.2—Equivalents of indices of refraction and butyro-refractometer readings

Refractive index (n_D)	Fourth decimal place of n_D									
	0	1	2	3	4	5	6	7	8	9
	SCALE READINGS									
1·422	0·0	0·1	0·2	0·4	0·5	0·6	0·7	0·9	1·0	1·1
1·423	1·2	1·4	1·5	1·6	1·7	1·9	2·0	2·1	2·2	2·4
1·424	2·5	2·6	2·7	2·8	3·0	3·1	3·2	3·3	3·5	3·6
1·425	3·7	3·8	4·0	4·1	4·2	4·3	4·5	4·6	4·7	4·8
1·426	5·0	5·1	5·2	5·4	5·5	5·6	5·7	5·9	6·0	6·1
1·427	6·2	6·4	6·5	6·6	6·8	6·9	7·0	7·1	7·2	7·4
1·428	7·5	7·6	7·7	7·9	8·0	8·1	8·2	8·4	8·5	8·6
1·429	8·7	8·9	9·0	9·1	9·2	9·4	9·5	9·6	9·8	9·9
1·430	10·0	10·1	10·3	10·4	10·5	10·6	10·7	10·9	11·0	11·1
1·431	11·3	11·4	11·5	11·6	11·8	11·9	12·0	12·2	12·3	12·4
1·432	12·5	12·7	12·8	12·9	13·0	13·2	13·3	13·5	13·6	13·7
1·433	13·8	14·0	14·1	14·2	14·4	14·5	14·6	14·7	14·9	15·0
1·434	15·1	15·3	15·4	15·5	15·6	15·8	15·9	16·0	16·2	16·3
1·435	16·4	16·6	16·7	16·8	17·0	17·1	17·2	17·4	17·5	17·6
1·436	17·8	17·9	18·0	18·2	18·3	18·4	18·5	18·7	18·8	18·9
1·437	19·1	19·2	19·3	19·5	19·6	19·7	19·8	20·0	20·1	20·3
1·438	20·4	20·5	20·6	20·8	20·9	21·1	21·2	21·3	21·4	21·6
1·439	21·7	21·8	22·0	22·1	22·2	22·4	22·5	22·6	22·7	22·9
1·440	23·0	23·2	23·3	23·4	23·5	23·7	23·8	23·9	24·1	24·2
1·441	24·3	24·5	24·6	24·7	24·8	25·0	25·1	25·2	25·4	25·5
1·442	25·6	25·8	25·9	26·1	26·2	26·3	26·5	26·6	26·7	26·9
1·443	27·0	27·1	27·3	27·4	27·5	27·7	27·8	27·9	28·1	28·2
1·444	28·3	28·5	28·6	28·7	28·9	29·0	29·2	29·3	29·4	29·6
1·445	29·7	29·9	30·0	30·1	30·3	30·4	30·6	30·7	30·8	30·9
1·446	31·1	31·2	31·4	31·5	31·6	31·8	31·9	32·1	32·2	32·3
1·447	32·5	32·6	32·8	32·9	33·0	33·2	33·3	33·5	33·6	33·7
1·448	33·9	34·0	34·2	34·3	34·4	34·6	34·7	34·9	35·0	35·1
1·449	35·3	35·4	35·6	35·7	35·8	36·0	36·1	36·3	36·4	36·5
1·450	36·7	36·8	37·0	37·1	37·2	37·4	37·5	37·7	37·8	37·9
1·451	38·1	38·2	38·3	38·5	38·6	38·7	38·9	39·0	39·2	39·3
1·452	39·5	39·6	39·7	39·9	40·0	40·1	40·3	40·4	40·6	40·7
1·453	40·9	41·0	41·1	41·3	41·4	41·5	41·7	41·8	42·0	42·1
1·454	42·3	42·4	42·5	42·7	42·8	43·0	43·1	43·3	43·4	43·6
1·455	43·7	43·9	44·0	44·2	44·3	44·4	44·6	44·7	44·9	45·0
1·456	45·2	45·3	45·5	45·6	45·7	45·9	46·0	46·2	46·3	46·4
1·457	46·6	46·7	46·9	47·0	47·2	47·3	47·5	47·6	47·7	47·9
1·458	48·0	48·2	48·3	48·5	48·6	48·8	48·9	49·1	49·2	49·4
1·459	49·5	49·7	49·8	50·0	50·1	50·2	50·4	50·5	50·7	50·8
1·460	51·0	51·1	51·3	51·4	51·6	51·7	51·9	52·0	52·2	52·3
1·461	52·5	52·7	52·8	53·0	53·1	53·3	53·4	53·6	53·7	53·9
1·462	54·0	54·2	54·3	54·5	54·6	54·8	55·0	55·1	55·3	55·4
1·463	55·6	55·7	55·9	56·0	56·2	56·3	56·5	56·6	56·8	56·9
1·464	57·1	57·3	57·4	57·6	57·7	57·9	57·0	58·2	58·3	58·5
1·465	58·6	58·8	58·9	59·1	59·2	59·4	59·5	59·7	59·8	60·0
1·466	60·2	60·3	60·5	60·6	60·8	60·9	61·1	61·2	61·4	61·5
1·467	61·7	61·8	62·0	62·2	62·3	62·5	62·6	62·8	62·9	63·1
1·468	63·2	63·4	63·5	63·7	63·8	64·0	64·2	64·3	64·5	64·7
1·469	64·8	65·0	65·1	65·3	65·4	65·6	65·7	65·9	66·1	66·2
1·470	66·4	66·5	66·7	66·8	67·0	67·2	67·3	67·5	67·7	67·8
1·471	68·0	68·1	68·3	68·4	68·6	68·7	68·9	69·1	69·2	69·4
1·472	69·5	69·7	69·9	70·0	70·2	70·3	70·5	70·7	70·8	71·0
1·473	71·1	71·3	71·4	71·6	71·8	71·9	72·1	72·2	72·4	72·5
1·474	72·7	72·9	73·0	73·2	73·3	73·5	73·7	73·8	74·0	74·1
1·475	74·3	74·5	74·6	74·8	75·0	75·1	75·3	75·5	75·6	75·8
1·476	76·0	76·1	76·3	76·5	76·7	76·8	77·0	77·2	77·3	77·5
1·477	77·7	77·9	78·1	78·2	78·4	78·6	78·7	78·9	79·1	79·2
1·478	79·4	79·6	79·8	80·0	80·1	80·3	80·5	80·6	80·8	81·0
1·479	81·2	81·3	81·5	81·7	81·9	82·0	82·2	82·4	82·5	82·7
1·480	82·9	83·1	83·2	83·4	83·6	83·8	83·9	84·1	84·3	84·5
1·481	84·6	84·8	85·0	85·2	85·3	85·5	85·7	85·9	86·0	86·2
1·482	86·4	86·6	86·7	86·9	87·1	87·3	87·5	87·6	87·8	88·0
1·483	88·2	88·3	88·5	88·7	88·9	89·1	89·2	89·4	89·6	89·8
1·484	90·0	90·2	90·3	90·5	90·7	90·9	91·1	91·2	91·4	91·6
1·485	91·8	92·0	92·1	92·3	92·5	92·7	92·9	93·0	93·2	93·4
1·486	93·6	93·8	94·0	94·1	94·3	94·5	94·7	94·8	95·0	95·2
1·487	95·4	95·6	95·8	96·0	96·1	96·3	96·6	96·7	96·9	97·0
1·488	97·2	97·4	97·6	97·8	98·0	98·1	98·3	98·5	98·7	98·[illegible]
1·489	99·1	99·2	99·4	99·6	99·8	100·0	—	—	—	—

Table A.3—Conversion of scale readings of dipping refractometer into refractive indices (prism 1)

Scale reading	$n_D = 1{\cdot}3$	Scale reading	$n_D = 1{\cdot}3$	Scale reading	$n_D = 1{\cdot}3$
− 5	2539	32	3972	69	5352
− 4	2578	33	4010	70	5388
− 3	2618	34	4048	71	5425
− 2	2657	35	4086	72	5461
− 1	2696	36	4124	73	5497
0	2736	37	4162	74	5533
1	2775	38	4199	75	5569
2	2814	39	4237	76	5606
3	2854	40	4275	77	5642
4	2893	41	4313	78	5678
5	2932	42	4350	79	5714
6	2971	43	4388	80	5750
7	3010	44	4426	81	5786
8	3049	45	4463	82	5822
9	3087	46	4500	83	5858
10	3126	47	4537	84	5894
11	3165	48	4575	85	5930
12	3204	49	4612	86	5966
13	3242	50	4650	87	6002
14	3281	51	4687	88	6038
15	3320	52	4724	89	6074
16	3358	53	4761	90	6109
17	3397	54	4798	91	6145
18	3435	55	4836	92	6181
19	3474	56	4873	93	6217
20	3513	57	4910	94	6252
21	3551	58	4947	95	6287
22	3590	59	4984	96	6323
23	3628	60	5021	97	6359
24	3667	61	5058	98	6394
25	3705	62	5095	99	6429
26	3743	63	5132	100	6464
27	3781	64	5169	101	6500
28	3820	65	5205	102	6535
29	3858	66	5242	103	6570
30	3896	67	5279	104	6605
31	3934	68	5316	105	6640

EXAMPLE: Scale division 8 corresponds to refractive index (n_D) = 1·33049.

Table A.4—Invert sugar table for 10 ml of Fehling's solution

(Lane and Eynon)

Ml of sugar solution required	Solutions containing besides invert sugar—									
	No sucrose		1 g sucrose per 100 ml		5 g sucrose per 100 ml		10 g sucrose per 100 ml		25 g sucrose per 100 ml	
	Invert sugar factor*	Mg invert sugar per 100 ml	Invert sugar factor*	Mg invert sugar per 100 ml	Invert sugar factor*	Mg invert sugar per 100 ml	Invert sugar factor*	Mg invert sugar per 100 ml	Invert sugar factor*	Mg invert sugar per 100 m
15	50·5	336	49·9	333	47·6	317	46·1	307	43·4	289
16	50·6	316	50·0	312	47·6	297	46·1	288	43·4	271
17	50·7	298	50·1	295	47·6	280	46·1	271	43·4	255
18	50·8	282	50·1	278	47·6	264	46·1	256	43·3	240
19	50·8	267	50·2	264	47·6	250	46·1	243	43·3	227
20	50·9	254·5	50·2	251·0	47·6	238·0	46·1	230·5	43·2	216
21	51·0	242·9	50·2	239·0	47·6	226·7	46·1	219·5	43·2	206
22	51·0	231·8	50·3	228·2	47·6	216·4	46·1	209·5	43·1	196
23	51·1	222·2	50·3	218·7	47·6	207·0	46·1	200·4	43·0	187
24	51·2	213·3	50·3	209·8	47·6	198·3	46·1	192·1	42·9	179
25	51·2	204·8	50·4	201·6	47·6	190·4	46·0	184·0	42·8	171
26	51·3	197·4	50·4	193·8	47·6	183·1	46·0	176·9	42·8	164
27	51·4	190·4	50·4	186·7	47·6	176·4	46·0	170·4	42·7	158
28	51·4	183·7	50·5	180·2	47·7	170·3	46·0	164·3	42·7	152
29	51·5	177·6	50·5	174·1	47·7	164·5	46·0	158·6	42·6	147
30	51·5	171·7	50·5	168·3	47·7	159·0	46·0	153·3	42·5	142
31	51·6	166·3	50·6	163·1	47·7	153·9	45·9	148·1	42·5	137
32	51·6	161·2	50·6	158·1	47·7	149·1	45·9	143·4	42·4	132
33	51·7	156·6	50·6	153·3	47·7	144·5	45·9	139·1	42·3	128
34	51·7	152·2	50·6	148·9	47·7	140·3	45·8	134·9	42·2	124
35	51·8	147·9	50·7	144·7	47·7	136·3	45·8	130·9	42·2	121
36	51·8	143·9	50·7	140·7	47·7	132·5	45·8	127·1	42·1	117
37	51·9	140·2	50·7	137·0	47·7	128·9	45·7	123·5	42·0	114
38	51·9	136·6	50·7	133·5	47·7	125·5	45·7	120·3	42·0	111
39	52·0	133·3	50·8	130·2	47·7	122·3	45·7	117·1	41·9	107
40	52·0	130·1	50·8	127·0	47·7	119·2	45·6	114·1	41·8	104
41	52·1	127·1	50·8	123·9	47·7	116·3	45·6	111·2	41·8	102
42	52·1	124·2	50·8	121·0	47·7	113·5	45·6	108·5	41·7	99
43	52·2	121·4	50·8	118·2	47·7	110·9	45·5	105·8	41·6	97
44	52·2	118·7	50·9	115·6	47·7	108·4	45·5	103·4	41·5	94
45	52·3	116·1	50·9	113·1	47·7	106·0	45·4	101·0	41·4	92
46	52·3	113·7	50·9	110·6	47·7	103·7	45·4	98·7	41·4	90
47	52·4	111·4	50·9	108·2	47·7	101·5	45·3	96·4	41·3	88
48	52·4	109·2	50·9	106·0	47·7	99·4	45·3	94·3	41·2	86
49	52·5	107·1	51·0	104·0	47·7	97·4	45·2	92·3	41·1	84
50	52·5	105·1	51·0	102·0	47·7	95·4	45·2	90·4	41·0	82

* Mg of invert sugar corresponding to 10 ml of Fehling's solution.

Table A.5—Invert sugar table for 25 ml of Fehling's solution (*Lane and Eynon*)

Ml of sugar solution required	Solutions containing besides invert sugar—			
	No sucrose		1 g sucrose per 100 ml	
	Invert sugar factor*	Mg invert sugar per 100 ml	Invert sugar factor*	Mg invert sugar per 100 ml
15	123·6	824	122·6	817
16	123·6	772	122·7	767
17	123·6	727	122·7	721
18	123·7	687	122·7	682
19	123·7	651	122·8	646
20	123·8	619·0	122·8	614·0
21	123·8	589·5	122·8	584·8
22	123·9	563·2	122·9	558·2
23	123·9	538·7	122·9	534·0
24	124·0	516·7	122·9	512·1
25	124·0	496·0	123·0	492·0
26	124·1	477·3	123·0	473·1
27	124·1	459·7	123·0	455·6
28	124·2	443·6	123·1	439·6
29	124·2	428·3	123·1	424·4
30	124·3	414·3	123·1	410·4
31	124·3	401·0	123·2	397·4
32	124·4	388·7	123·2	385·0
33	124·4	377·0	123·2	373·4
34	124·5	366·2	123·3	362·6
35	124·5	355·8	123·3	352·3
36	124·6	346·1	123·3	342·5
37	124·6	336·8	123·4	333·5
38	124·7	328·1	123·4	324·7
39	124·7	319·7	123·4	316·4
40	124·8	311·9	123·4	308·6
41	124·8	304·4	123·5	301·2
42	124·9	297·3	123·5	294·1
43	124·9	290·5	123·5	287·3
44	125·0	284·1	123·6	280·9
45	125·0	277·9	123·6	274·7
46	125·1	272·0	123·6	268·7
47	125·1	266·3	123·7	263·1
48	125·2	260·8	123·7	257·7
49	125·2	255·5	123·7	252·5
50	125·3	250·6	123·8	247·6

* Mg of invert sugar corresponding to 25 ml of Fehling's solution.

Table A.6—Dextrose table (*Lane and Eynon*)
(All figures relate to anhydrous dextrose)

Ml of sugar solution required	For 10 ml of Fehling's solution		For 25 ml of Fehling's solution	
	Dextrose factor*	Mg dextrose per 100 ml	Dextrose factor†	Mg dextrose per 100 ml
15	49·1	327	120·2	801
16	49·2	307	120·2	751
17	49·3	289	120·2	707
18	49·3	274	120·2	668
19	49·4	260	120·3	633
20	49·5	247·4	120·3	601·5
21	49·5	235·8	120·3	572·9
22	49·6	225·5	120·4	547·3
23	49·7	216·1	120·4	523·6
24	49·8	207·4	120·5	501·9
25	49·8	199·3	120·5	482·0
26	49·9	191·8	120·6	463·7
27	49·9	184·9	120·6	446·8
28	50·0	178·5	120·7	431·1
29	50·0	172·5	120·7	416·4
30	50·1	167·0	120·8	402·7
31	50·2	161·8	120·8	389·7
32	50·2	156·9	120·8	377·6
33	50·3	152·4	120·9	366·3
34	50·3	148·0	120·9	355·6
35	50·4	143·9	121·0	345·6
36	50·4	140·0	121·0	336·3
37	50·5	136·4	121·1	327·4
38	50·5	132·9	121·2	318·8
39	50·6	129·6	121·2	310·7
40	50·6	126·5	121·2	303·1
41	50·7	123·6	121·3	295·9
42	50·7	120·8	121·4	289·0
43	50·8	118·1	121·4	282·4
44	50·8	115·5	121·5	276·1
45	50·9	113·0	121·5	270·1
46	50·9	110·6	121·6	264·3
47	51·0	108·4	121·6	258·8
48	51·0	106·2	121·7	253·5
49	51·0	104·1	121·7	248·4
50	51·1	102·2	121·8	243·6

* Mg of dextrose corresponding to 10 ml of Fehling's solution.
† Mg of dextrose corresponding to 25 ml of Fehling's solution.

Table A.7—Laevulose table (*Lane and Eynon*)
(All figures relate to anhydrous laevulose)

Ml of sugar solution required	For 10 ml of Fehling's solution		For 25 ml of Fehling's solution	
	Laevulose factor*	Mg laevulose per 100 ml	Laevulose factor†	Mg laevulose per 100 ml
15	52·2	348	127·4	849
16	52·3	327	127·4	796
17	52·3	308	127·5	750
18	52·4	291	127·5	708
19	52·5	276	127·6	672
20	52·5	262·5	127·6	638·0
21	52·6	250·6	127·7	608·1
22	52·7	239·6	127·7	580·6
23	52·7	229·1	127·8	555·5
24	52·8	220·0	127·8	532·5
25	52·8	211·3	127·9	511·5
26	52·9	203·3	127·9	491·9
27	52·9	196·0	128·0	474·0
28	53·0	189·3	128·0	457·2
29	53·1	183·1	128·1	441·6
30	53·2	177·2	128·1	427·0
31	53·2	171·7	128·1	413·3
32	53·3	166·5	128·2	400·5
33	53·3	161·6	128·2	388·5
34	53·4	157·0	128·3	377·3
35	53·4	152·6	128·3	366·7
36	53·5	148·6	128·4	356·6
37	53·5	144·7	128·4	347·0
38	53·6	140·9	128·5	338·1
39	53·6	137·3	128·5	329·6
40	53·6	134·0	128·6	321·5
41	53·7	130·9	128·6	313·7
42	53·7	127·9	128·6	306·2
43	53·8	125·1	128·7	299·2
44	53·8	122·4	128·7	292·5
45	53·9	119·8	128·8	286·2
46	53·9	117·2	128·8	280·0
47	53·9	114·7	128·9	274·2
48	54·0	112·4	128·9	268·6
49	54·0	110·2	129·0	263·2
50	54·0	108·0	129·0	258·0

* Mg of laevulose corresponding to 10 ml of Fehling's solution.
† Mg of laevulose corresponding to 25 ml of Fehling's solution.

Table A.8—Maltose table (*Lane and Eynon*)

Ml of sugar solution required	For 10 ml of Fehling's solution				For 25 ml of Fehling's solution			
	Hydrated maltose $C_{12}H_{22}O_{11}, H_2O$		Anhydrous maltose $C_{12}H_{22}O_{11}$		Hydrated maltose $C_{12}H_{22}O_{11}, H_2O$		Anhydrous maltose $C_{12}H_{22}O_{11}$	
	Factor*	Mg per 100 ml	Factor*	Mg per 100 ml	Factor†	Mg per 100 ml	Factor†	Mg per 100 ml
15	81·3	542	77·2	515	208·2	1388	197·8	1319
16	81·2	507	77·1	482	207·8	1298	197·4	1233
17	81·1	477	77·0	453	207·4	1220	197·0	1159
18	81·0	450	77·0	427	207·1	1151	196·7	1093
19	80·9	426	76·9	405	206·8	1088	196·5	1034
20	80·8	404·0	76·8	383·8	206·5	1032·3	196·2	980·7
21	80·7	384·3	76·7	365·1	206·1	981·6	195·8	932·5
22	80·6	366·4	76·6	348·1	205·8	935·5	195·5	888·7
23	80·5	350·0	76·5	332·5	205·4	893·2	195·1	848·5
24	80·4	335·0	76·4	318·3	205·1	854·5	194·8	811·8
25	80·4	321·5	76·4	305·4	204·8	819·0	194·5	778·1
26	80·3	308·8	76·3	293·4	204·4	786·3	194·2	747·0
27	80·2	297·0	76·2	282·2	204·1	756·0	193·9	718·2
28	80·1	286·1	76·1	271·8	203·8	727·9	193·6	691·5
29	80·0	276·0	76·0	262·2	203·5	701·7	193·3	666·6
30	80·0	266·6	76·0	253·3	203·2	677·3	193·0	643·4
31	79·9	257·8	75·9	244·9	202·9	654·3	192·8	621·6
32	79·9	249·7	75·9	237·2	202·6	633·1	192·5	601·4
33	79·8	241·9	75·8	229·8	202·3	613·0	192·2	582·4
34	79·8	234·6	75·8	222·9	202·0	594·3	191·9	564·6
35	79·7	227·6	75·7	216·2	201·8	576·5	191·7	547·7
36	79·6	221·1	75·6	210·0	201·5	559·7	191·4	531·7
37	79·6	215·0	75·6	204·3	201·2	543·9	191·2	516·7
38	79·5	209·2	75·5	198·7	201·0	528·9	191·0	502·5
39	79·5	203·8	75·5	193·6	200·8	514·7	190·8	489·0
40	79·4	198·5	75·4	188·6	200·5	501·3	190·5	476·2
41	79·4	193·7	75·4	184·3	200·3	488·5	190·3	464·1
42	79·3	188·8	75·3	179·4	200·1	476·3	190·1	452·5
43	79·3	184·3	75·3	175·1	199·8	464·7	189·8	441·5
44	79·2	180·0	75·2	171·0	199·6	453·6	189·6	430·9
45	79·2	175·9	75·2	167·1	199·4	443·0	189·4	420·9
46	79·1	172·0	75·1	163·4	199·2	433·1	189·2	411·4
47	79·1	168·3	75·1	159·9	199·0	423·6	189·0	402·4
48	79·1	164·7	75·1	156·5	198·9	414·4	188·9	393·7
49	79·0	161·2	75·0	153·1	198·7	405·5	188·8	385·2
50	79·0	158·0	75·0	150·1	198·6	397·2	188·7	377·3

* Mg of maltose corresponding to 10 ml of Fehling's solution.
† Mg of maltose corresponding to 25 ml of Fehling's solution.

Table A.9—Lactose table (*Lane and Eynon*)

Ml of sugar solution required	For 10 ml of Fehling's solution				For 25 ml of Fehling's solution			
	Hydrated lactose $C_{12}H_{22}O_{11}, H_2O$		Anhydrous lactose $C_{12}H_{22}O_{11}$		Hydrated lactose $C_{12}H_{22}O_{11}, H_2O$		Anhydrous lactose $C_{12}H_{22}O_{11}$	
	Factor*	Mg per 100 ml	Factor*	Mg per 100 ml	Factor†	Mg per 100 ml	Factor†	Mg per 100 ml
15	68·3	455	64·9	432	172·5	1150	163·9	1093
16	68·2	426	64·8	405	172·1	1076	163·5	1022
17	68·2	401	64·8	381	171·7	1010	163·1	960
18	68·1	378	64·7	359	171·4	952	162·8	906
19	68·1	358	64·7	340	171·1	900	162·5	855
20	68·0	340·0	64·6	323·0	170·9	854·6	162·3	811·8
21	68·0	323·8	64·6	307·6	170·6	812·4	162·0	772·3
22	68·0	309·1	64·6	293·6	170·4	774·5	161·8	735·8
23	67·9	295·4	64·5	280·6	170·2	740·0	161·6	703·0
24	67·9	282·9	64·5	268·8	170·0	708·5	161·5	673·1
25	67·9	271·6	64·5	258·0	169·9	679·5	161·4	645·5
26	67·9	261·0	64·5	248·0	169·7	652·7	161·2	620·1
27	67·8	251·1	64·4	238·5	169·5	627·9	161·0	596·5
28	67·8	242·1	64·4	230·0	169·3	604·8	160·8	574·6
29	67·8	233·8	64·4	222·2	169·2	583·3	160·7	554·1
30	67·8	226·0	64·4	214·7	169·0	563·3	160·6	535·1
31	67·8	218·7	64·4	207·8	168·9	544·8	160·5	517·6
32	67·8	211·9	64·4	201·3	168·8	527·4	160·4	501·0
33	67·8	205·6	64·4	195·3	168·6	511·0	160·2	485·5
34	67·9	199·7	64·5	189·7	168·5	495·6	160·1	470·8
35	67·9	194·0	64·5	184·3	168·4	481·1	160·0	457·0
36	67·9	188·6	64·5	179·2	168·2	467·3	159·8	443·9
37	67·9	183·5	64·5	174·3	168·1	454·3	159·7	431·6
38	67·9	178·7	64·5	169·8	168·0	442·1	159·6	420·0
39	67·9	174·1	64·5	165·4	167·9	430·5	159·5	409·0
40	67·9	169·7	64·5	161·2	167·8	419·5	159·4	398·5
41	68·0	165·9	64·6	157·6	167·7	409·0	159·3	388·6
42	68·0	161·9	64·6	153·8	167·6	399·1	159·2	379·1
43	68·0	158·1	64·6	150·2	167·6	389·7	159·2	370·2
44	68·0	154·7	64·6	147·0	167·5	380·7	159·1	361·7
45	68·1	151·3	64·7	143·7	167·4	372·1	159·0	353·5
46	68·1	148·0	64·7	140·6	167·4	363·9	159·0	345·7
47	68·2	145·1	64·8	137·8	167·3	356·0	158·9	338·2
48	68·2	142·1	64·8	135·0	167·2	348·3	158·8	330·9
49	68·2	139·2	64·8	132·2	167·2	341·0	158·8	324·0
50	68·3	136·6	64·9	129·8	167·1	334·2	158·7	317·5

* Mg of lactose corresponding to 10 ml of Fehling's solution.
† Mg of lactose corresponding to 25 ml of Fehling's solution.

Table A.10—Quantities of copper and copper oxide produced under standard conditions by various carbohydrates*

(Quantities expressed in milligrams in all cases)

Cupric oxide	Cuprous oxide	Copper	Dextrose	Starch	Laevulose	Hydrated lactose $C_{12}H_{22}O_{11}, H_2O$	Anhydrous lactose	Maltose	Invert sugar	Cane sugar
100	89·9	79·9	—	—	—	59·2	56·2	72·5	—	—
110	98·9	87·9	—	—	—	65·2	61·9	79·8	45·3	43·0
120	107·9	95·9	46·5	41·8	51·7	71·2	67·6	87·2	49·2	46·7
130	116·9	103·9	50·4	45·4	55·5	77·2	73·3	95·3	53·1	50·4
140	125·9	111·9	54·2	48·8	59·5	83·2	79·0	102·0	57·0	54·2
150	134·9	119·8	58·0	52·2	63·9	89·3	84·8	109·2	61·0	58·0
160	143·9	127·8	61·8	55·6	68·1	95·4	90·6	116·8	65·1	61·8
170	152·9	135·8	65·7	59·1	72·4	101·4	96·3	124·2	69·2	65·7
180	161·9	143·8	69·6	62·6	76·7	107·4	102·0	131·5	73·4	69·7
190	170·9	151·8	73·6	66·2	80·9	113·5	107·9	138·8	77·5	73·6
200	179·9	159·8	77·6	69·8	85·3	119·7	113·7	146·3	81·5	77·4
210	188·9	167·8	81·6	73·4	89·5	126·0	119·7	153·6	85·7	81·4
220	197·9	175·8	85·5	77·0	94·0	132·2	125·6	161·0	90·0	85·5
230	206·9	183·8	90·0	81·0	98·6	138·4	131·5	168·3	94·4	89·7
240	215·9	191·7	94·2	84·8	103·0	144·6	137·4	175·7	98·7	93·8
250	224·9	199·7	98·3	88·5	107·3	150·7	143·2	183·1	102·9	97·8
260	233·9	207·7	102·5	92·3	111·8	157·0	149·2	190·5	107·1	101·7
270	242·9	215·7	106·7	96·0	116·4	163·0	154·9	197·9	111·4	105·8
280	251·9	223·7	110·8	99·7	121·0	169·2	160·7	205·2	115·7	109·9
290	260·8	231·7	115·0	103·5	125·6	175·5	166·7	212·6	120·1	114·1
300	269·8	239·7	119·4	107·5	130·1	182·3	173·2	220·0	124·6	118·4
310	278·8	247·7	123·7	111·3	134·7	188·9	179·5	227·3	129·1	122·6
320	287·8	255·7	128·2	115·4	139·5	195·4	185·6	234·7	133·7	127·0
330	296·8	263·6	132·6	119·3	144·5	202·0	191·9	242·1	138·2	131·3
340	305·8	271·6	137·1	123·4	149·5	208·7	198·3	249·5	142·8	135·7
350	314·8	279·6	141·7	127·5	154·2	215·3	204·5	256·9	147·7	140·3
360	323·8	287·6	146·4	131·8	158·9	222·0	210·9	264·3	152·7	145·1
370	332·8	295·6	151·2	136·1	163·8	228·6	217·2	271·6	157·5	149·6
380	341·8	303·6	155·6	140·1	168·5	235·2	223·4	279·0	161·9	153·8
390	350·8	311·6	160·5	144·5	173·5	241·9	229·8	286·4	166·5	158·2
400	359·8	319·6	165·2	148·7	178·4	248·6	236·2	293·7	171·4	162·8
410	368·8	327·6	170·1	153·1	183·3	254·7	242·0	301·1	176·2	167·2
420	377·8	335·5	175·0	157·5	188·3	260·9	247·9	—	181·2	172·1
430	386·8	343·5	179·9	161·9	193·2	267·2	253·8	—	186·2	176·9
440	395·8	351·5	185·0	166·5	198·4	273·8	260·1	—	191·2	181·7
450	404·8	359·5	190·0	171·0	203·6	280·4	266·4	—	196·3	186·5
460	413·8	367·5	195·0	175·5	208·8	287·2	272·8	—	201·6	191·5

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* For extended tables, see Elsdon, *Analyst*, 1923, **48**, 435.

ABBREVIATIONS USED IN THE REFERENCES

Advanc. Protein Chem.	Advances in Protein Chemistry
Amer. J. Hyg.	American Journal of Hygiene
Amer. J. Physiol.	American Journal of Physiology
Amer. J. Publ. Hlth.	American Journal of Public Health
Ann. Chim. anal.	Annales de Chimie analytique et de Chimie appliquée
Ann. Falsif.	Annales des Falsifications
Ann. Rep. Lancs County Anal.	Annual Report of the Lancashire County Analyst
A.O.A.C.	Official and Tentative Methods of the Association of Official Agricultural Chemists, Washington
Arch. Biochem.	Archives of Biochemistry and Biophysics
Arch. exper. Pathol. Pharm.	Archiv für experimentelle Pathologie und Pharmakologie
Arch. Ges. Virusforsch.	Archiv für die gesamte Virusforschung (Vienna)
Arch. Hyg.	Archiv der Hygiene und Bakteriologie
Aust. J. Dairy Tech.	Australian Journal of Dairy Technology
B.C.A.	British Chemical (and Physiological) Abstracts
Biochem. J.	Biochemical Journal
Biochem. Z.	Biochemische Zeitschrift
B.I.O.S.	British Intelligence Objectives Sub-committee
B.P.	British Pharmacopœia
Brit. Food J.	British Food Journal
Brit. J. Nutr.	British Journal of Nutrition
Brit. Med. Bull.	British Medical Bulletin
Brit. Med. J.	British Medical Journal
Bull. Soc. Chim.	Bulletin de la Société chimique de France
Bull. Soc. Chim. biol.	Bulletin de la Société de Chimie biologique
C.A.	Chemical Abstracts (of American Chemical Society)
Canad. J. Res.	Canadian Journal of Research (Ottawa)
Cereal Chem.	Cereal Chemistry (St. Paul, Minn.)
Chem. and Ind.	Chemistry and Industry
Chem. Fabr.	Chemische Fabrik (Berlin)
Chem. News	Chemical News
Chem. Weekblad	Chemisch Weekblad
Chem. Zbl.	Chemisches Zentralblatt
Chim. et Ind.	Chimie et Industrie
C.R. Acad. Agric. France	Comptes rendus hebdomadaires des Séances de l'Académie d'Agriculture de France
C.R. Acad. Sci. Paris	Comptes rendus hebdomadaires des Séances de l'Académie des Sciences, Paris
C.R. Soc. Biol. Paris	Comptes rendus de la Société de Biologie, Paris
Dairy Ind.	Dairy Industries
D.S.A.	Dairy Science Abstracts
Fermentforsch.	Fermentforschung
Fmg. S. Africa	Farming in South Africa
Food Ind.	Food Industries (N.Y.)
Food Inv. Bd. Spec. Rep.	Food Investigation Board Special Report
Food Manuf.	Food Manufacture
Food Res.	Food Research (Chicago)
Forsch. ü. Lebensmitt.	Forschungen über Lebensmitteln
Helv. Chim. Act.	Helvetica chimica acta
I.D.C.	International Dairy Congress
Ill. Agric. Exp. Sta. Bull.	Illinois University Agricultural Experiment Station

Imp. Bur. Dairy Sci. Tech. Com.	Imperial Bureau of Dairy Science, Technical Communication
Ind. J. Dairy Sci.	Indian Journal of Dairy Science
Ind. J. Vet. Sci.	Indian Journal of Veterinary Science and Animal Husbandry
J. Agric. Res.	Journal of Agricultural Research (Washington)
J. Agric. Sci.	Journal of Agricultural Science
J. Amer. Chem. Soc.	Journal of the American Chemical Society
J. Amer. Vet. Med. Ass.	Journal of the American Veterinary Medical Association
J.A.O.A.C.	Journal of the Association of Official Agricultural Chemists (Washington)
J. Bact.	Journal of Bacteriology (Baltimore)
J. Biol. Chem.	Journal of Biological Chemistry (Baltimore)
J. Chem. Soc.	Journal of the Chemical Society
J. Coun. Sci. Industr. Res. Aust.	Journal of the Council of Scientific and Industrial Research, Australia
J. Dairy Res.	Journal of Dairy Research
J. Dairy Sci.	Journal of Dairy Science (Baltimore)
J. Dept. Agric. Eire	Journal of the Department of Agriculture, Eire
J. Ind. Eng. Chem.	Journal of Industrial and Engineering Chemistry (Easton, Pa.)
J. Min. Agric.	Journal of the Ministry of Agriculture and Fisheries
J. Nutr.	Journal of Nutrition (Baltimore)
J. Pharm. Chim.	Journal de Pharmacie et de Chimie
J. Phys. Coll. Chem.	Journal of Physical and Colloid Chemistry
J. Physiol.	Journal of Physiology
J. Roy. San. Inst.	Journal of the Royal Sanitary Institute
J. Roy. Soc. Arts	Journal of the Royal Society of Arts
J. Sci. Food Agric.	Journal of the Science of Food and Agriculture
J. Soc. Chem. Ind.	Journal of the Society of Chemical Industry
J. Soc. Dairy Tech.	Journal of the Society of Dairy Technology
Landw. Jb. Schweiz	Landwirtschaftliches Jahrbuch der Schweiz
Landw. Versuchsstat.	Die landwirtschaftlichen Versuchsstationen
Les Nouv. chimiques	Les Nouvelles chimiques
Milchw. Forsch.	Milchwirtschaftliche Forschungen
Milchw. Zbl.	Milchwirtschaftliches Zentralblatt
Milk Dlr.	Milk Dealer (Milwaukee, Wis., U.S.A.)
Milk Ind.	Milk Industry
Milk Ind. Fdn. Conv. Proc.	Milk Industry Foundation Convocation Proceedings (U.S.A.)
Milk Pl. Mth.	Milk Plant Monthly (Chicago)
Molkereiztg Hildesh.	Molkereizeitung (Hildesheim)
Nat. Butt. Cheese J.	National Butter and Cheese Journal (Milwaukee)
Neth. Milk Dairy J.	Netherlands Milk and Dairy Journal (Amsterdam)
Nutr. Abstr. Rev.	Nutritional Abstracts and Reviews
N.Z.J. Sci. Tech.	New Zealand Journal of Science and Technology
Pharm. Weekblad	Pharmaceutisch Weekblad
Pharm. Zent.	Pharmazeutische Zentralhalle
Proc. Amer. Dairy Sci. Ass. W. Div.	Proceedings of the American Dairy Science Association, Western Division
Proc. Roy. Soc.	Proceedings of the Royal Society
Proc. Roy. Soc. Edin.	Proceedings of the Royal Society of Edinburgh
Proc. Soc. Agric. Bact.	Proceedings of the Society of Agricultural Bacteriologists (now the Society for Applied Bacteriology)
Proc. Soc. Exp. Biol. N.Y.	Proceedings of the Society for Experimental Biology and Medicine, New York.

Rep. Dep. Sci. Ind. Res.	Report of the Department of Scientific and Industrial Research, London
Rep. Food Inv. Bd.	Report of the Food Investigation Board, London
Rep. Pub. Hlth Med. Subj.	Reports on Public Health and Medical Subjects, Ministry of Health
Sci. Agric.	Scientific Agriculture (Ottawa)
Sci. Proc. R. Dublin Soc.	Scientific Proceedings of the Royal Dublin Society
Scot. J. Agric.	Scottish Journal of Agriculture
Spec. Rep. Ser. M.R.C.	Special Report Series, Medical Research Council
Tabul. biol. Berlin	Tabulae biologicae periodicae (Berlin)
Trans. Highland Agric. Soc.	Transactions of the Highland Agricultural Society
Vet. Rec.	Veterinary Record
Welsh J. Agric.	Welsh Journal of Agriculture
Y.B.P.	Yearbook of Pharmacy
Z. anal. Chem.	Zeitschrift für analytische Chemie
Z. Fleisch. Milchhyg.	Zeitschrift für Fleisch- und Milchhygiene
Z. Lebensm. Unters. Forsch.	Zeitschrift für Lebensmittel-Untersuchung und Forschung
Z. Unters. Lebensm.	Zeitschrift für Untersuchung der Lebensmittel
Z. Unters. Nahr. Genussm.	Zeitschrift für Untersuchung der Nahrungs- und Genussmittel

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